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Short communication

SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats

Aron H. Lichtman *

Department of Pharmacology and Toxicology, MCV Campus, P.O. Box 980613, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298-0613 USA

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Abstract

A tonically active endogenous cannabinoid system has been proposed to modulate learning and memory. The purpose of the present study was to determine whether administration of the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A) would enhance memory as assessed in an eight-arm radial maze task. Because the high degree of choice accuracy in the standard radial-arm maze procedure precludes the possibility of detecting memory enhancement, the difficulty of the task was increased by imposing a delay of varying durations between a two-phase procedure consisting of acquisition and test phases. Significantly fewer errors were committed during the test phase following an injection of SR 141716A than the vehicle treatment. These results provide additional evidence supporting the hypothesis that endogenous cannabinoid systems play a role in memory processes.

Keywords: Radial-arm maze; SR141716A; Cannabinoid; Working memory

1. Introduction

Administration of cannabinoid agonists has been well documented to impair memory in operant (Heyser et al., 1993; Nakamura et al., 1991), radial-arm maze (Heyser et al., 1993; Nakamura et al., 1991) and Morris water maze (Ferrari et al., 1999) tasks in rodents. The high concentration of cannabinoid receptors (Herkenham et al., 1991) and presence of the endogenous cannabinoid anandamide (Felder et al., 1996) in the hippocampus, a brain area associated with working memory, suggest that a cannabinoid neurochemical system may play a modulatory role in learning and memory (Hampson and Deadwyler, 1998). Evidence consistent with such a proposal is that administration of anandamide impaired memory as assessed in a nonmatch-to-position operant task in rats pretreated with the protease inhibitor phenylmethylsulfonyl fluoride (Mallet and Beninger, 1996). Similarly, the stable synthetic analog, *R*-methanandamide impaired accuracy in a repeated acquisition operant task (Brodkin and Moerschbaecher, 1997).

Tonic activation of a cannabinoid system has been speculated to play a role in an active forgetting process in which extraneous information is deleted from memory storage (Herkenham et al., 1991). In support of this hypothesis, the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A) given alone enhanced memory as assessed in a social recognition memory task in mice and rats (Terranova et al., 1996). Moreover, cannabinoid CB_1 receptor knock-out mice retained memory longer than wild type controls in a two-trial object recognition test (Reibaud et al., 1999). Conversely, SR 141716A failed to enhance memory as assessed in fixed consecutive number responding in pigeons (Mansbach et al., 1996), in an operant repeated acquisition procedure in rats (Brodkin and Moerschbaecher, 1997), and in a nonmatch-to-position task in rats (Mallet and Beninger, 1998). These behavioral studies taken together suggest that blocking cannabinoid transmission is more likely to enhance memory that persists for minutes or hours than memory that is prone to a rapid rate of forgetting (i.e. less than 10 s), such as that found in the operant tasks.

The primary objective of the present study was to determine whether SR 141716A can improve choice accu-

* Tel.: +1-804-828-8480; fax: +1-804-828-2117.
E-mail address: alichtma@hsc.vcu.edu (A.H. Lichtman).

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racy in the radial-arm maze, a spatial memory task (Olton, 1987) that has been particularly sensitive to the memory-disruptive effects of cannabinoids (Lichtman et al., 1995). Well-trained rats exhibit a high degree of choice accuracy in the standard radial-arm maze task, thus precluding the possibility of enhancing performance. Therefore, the possibility of observing a drug-induced improvement in choice accuracy was optimized by employing a two-phase procedure, separated by a delay of varying durations to increase the difficulty of the task. This type of procedure has been previously reported to increase the number of errors committed in the radial arm maze (Martin et al., 1992; Pilcher et al., 1997).

2. Materials and methods

2.1. Subjects

The subjects were 10 Sprague–Dawley (Harlan, IN) male rats that were individually housed in a temperature-controlled (20–22°C) environment, with a 12-h light/dark cycle. Subjects were placed on a food-restricted diet in order to maintain a weight between 290 and 300 g, approximately 85% of their free-feeding weight. Each rat was given between 12 and 16 g of food (Prolab, Agway, Richmond, VA) rations/day, and water was available ad libitum at all times except during the test session.

2.2. Drugs

SR 141716A (National Institute on Drug Abuse, Rockville, MD) was dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and diluted with saline in a final ratio of 1:1:18 (ethanol/alkamuls/saline). All injections were given through the i.p. route of administration in a volume of 1 ml/kg.

2.3. Apparatus and training

The radial-arm maze and initial training procedure were identical to that previously described (Lichtman and Martin, 1996). Each of the eight arms was baited with a 45-mg rodent grain-base formula, dustless precision pellets (Bioserve, Frenchtown, NJ) placed 5 cm from the end. After a subject visited each arm and obtained all the food pellets, with one or no re-entries into a previously visited arm on three consecutive sessions, it was trained in an adapted version of the task that incorporated two phases with a delay of varying durations between each phase. During the acquisition phase, one of the arms was randomly selected and a Plexiglas barricade blocked its entryway. Each of the available seven arms was baited with a food pellet prior to the subject's placement in the maze. After the subject entered the seven available arms and consumed all the food pellets, it was removed from the maze. Following a delay of varying durations, the subject was returned to the maze for the test phase. During this phase, all eight arms were available; however, only the previously unavailable arm was baited with a food pellet. The number of entries and the duration of time required for each subject to enter the previously blocked arm and consume the food pellet was recorded.

The dose SR 141716A selected was 3 mg/kg, an amount slightly higher than its AD50 in antagonizing the disruptive effects of Δ^9-tetrahydrocannabinol in radial-arm maze choice accuracy (Lichtman and Martin, 1996). In the first experiment, vehicle or SR 141716A was given 20 min prior to acquisition and the delay between the two phases was 20 min or 1, 2, 4, 6, or 24 h. In the second experiment, a 6-h delay was imposed between the two phases. Each rat received two injections of vehicle or SR 141716A, with each respective injection given 20 min before each phase. In the third experiment, vehicle or SR 141716A was given immediately after acquisition, then testing occurred after a 6-h delay. In each experiment, the treatments were counter-balanced to control for any order effects. Subjects were given a maximum of two tests/week.

2.4. Statistical analysis

An observer scored the number of correct responses and errors (i.e. re-entries) committed by each rat and the amount of time taken to obtain all the available food pellets for each phase. The dependent measures of interest were the number of errors committed as well the amount of time it took each rat to enter an arm. Two-way within subject analysis of variances (ANOVAs) were used to analyze each dependent measure in experiments 1 and 2, and a paired *t*-test was employed in the third experiment. Differences were considered significant at the $P < 0.05$ level.

3. Results

In order to determine whether a single dose of SR 141716A could diminish delay-induced errors, subjects were administered either vehicle or drug 20 min prior to the acquisition phase. Subjects were highly proficient during acquisition, entering each of the available seven arms with virtually no errors and taking approximately 12 s/arm regardless of drug condition (data not shown). After completing this phase, the subjects were removed from the maze, returned to their home cages, and placed back in the maze for the test phase following a delay of 20 min or 1, 2, 4, 6, or 24 h. A significant main effect of SR 141716A in reducing the number of errors committed during the test phase was found, $F(1,9) = 14$, $P < 0.05$ (Fig. 1, top panel). A significant main effect of time, $F(5,45) = 18$, $P < 0.05$, was also found indicating that the number of errors in-

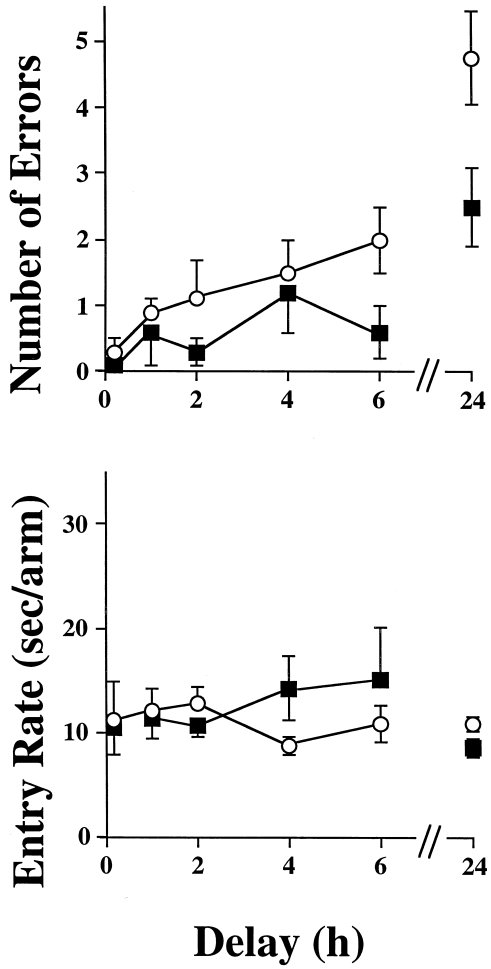

Fig. 1. The effects of SR 141716A on radial-arm maze performance during the test phase following delays of varying durations. Subjects were given an i.p. injection of vehicle (○) or 3 mg/kg of SR 141716A (■) 20 min prior to the acquisition phase. All results are presented as means ± S.E., $n = 10$ rats/group. Top panel: SR 141716A given before the acquisition phase significantly decreased the number of errors committed during the test phase. Bottom panel: The entry rate into each arm was unaffected by either the drug treatment or the delay duration.

creased with increasing delays. The interaction between time and SR 141716A did not achieve statistical significance, $F(5,45) = 1$, $P = 0.4$. The rate of entry into each arm was unaffected by drug or delay (Fig. 1, bottom panel; $P > 0.50$).

The purpose of the second experiment was to determine whether the enhancing effects of SR 141716A depended on whether it was injected before the acquisition or test phase. Given the relatively good performance exhibited by the subjects when the delay was 4 h or less (i.e. less than 1.5 errors committed), a 6-h delay was employed in experiment 2 to increase the difficulty of the task. Once again, the drug failed to affect performance during acquisition, virtually no errors were committed and the rate of entry into each arm was approximately 14 s/arm regardless of condition (data not shown). SR 141716A only reduced the number of errors committed during the test phase when it was given 20 min prior to acquisition, as indicated by a significant effect of the acquisition phase injection, $F(1,19) = 5.9$, $P < 0.05$ (Fig. 2, top panel). The drug had no impact when it was given 20 min prior to the test phase as indicated by lack of significance for the test phase injection main effect and interaction between the acquisition and test phase injections ($P > 0.8$ for each). Again, the rate of entry into each arm was unaffected by drug or delay (Fig. 2, bottom panel; $P > 0.50$). The large variability in the double injection of SR 141716A condition resulted from one rat that made no errors, but entered the correct arm 2 min after placement in the maze.

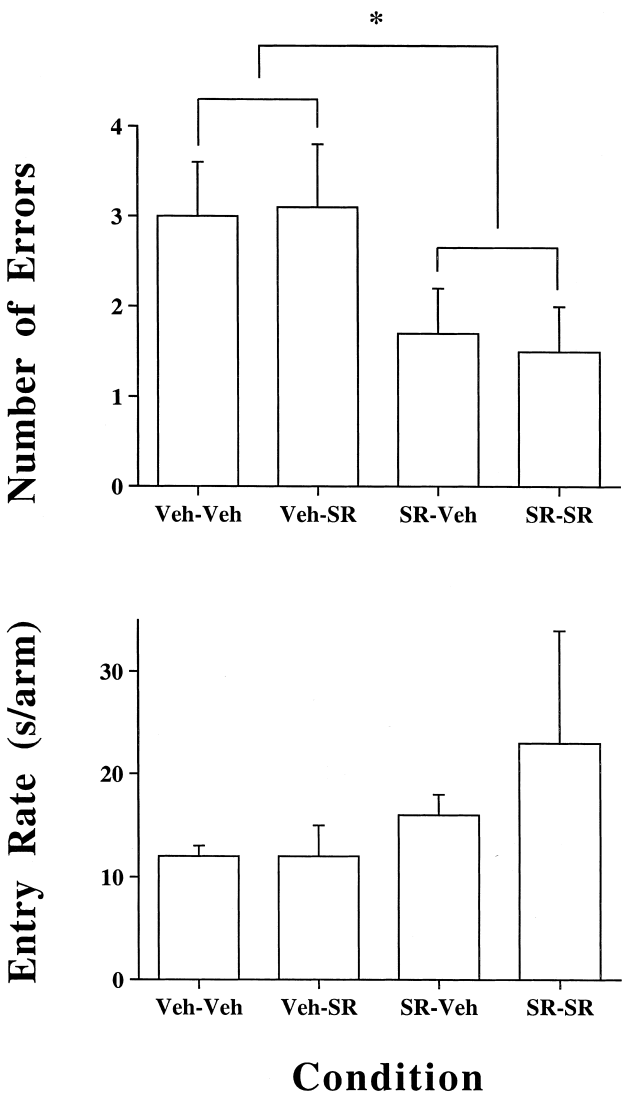

Fig. 2. Radial-arm maze performance during the test phase following a 6-h delay. Subjects were given an i.p. injection of vehicle or 3 mg/kg of SR 141716A 20 min before the acquisition phase and then given a second i.p. injection of either vehicle or 3 mg/kg of SR 141716A 20 min before the test phase. All results are presented as means ± S.E., $n = 10$ rats/group. Top panel: SR 141716A given before the acquisition phase significantly decreased the number of errors during the test phase, regardless of the test phase injection (*ANOVA, $P < 0.05$). Bottom panel: The entry rate into each arm was unaffected by drug treatment.

One explanation accounting for the failure of SR 141716A given 20 min before the test phase to enhance performance is that a longer pretreatment period (e.g. 6 h) is required. Therefore, a final experiment was conducted in which subjects were given an injection of vehicle or SR 141716A immediately after the acquisition phase and evaluated in the test phase 6 h later. During the test phase, there were no significant differences between vehicle and SR 141716A treatment for either the number of errors committed ($P = 0.50$) or entry rate ($P = 0.12$). Subjects committed 3.2 ± 0.7 errors and had a rate of 11 ± 1 s/arm under the vehicle condition, and committed 2.7 ± 1.6 errors and had a rate of 13 ± 1.2 s/arm when administered SR 141716A.

4. Discussion

Increasing the difficulty of an adapted radial-arm maze procedure, by imposing a delay between the two phases, led to a decrease in choice accuracy during the test phase that was ameliorated by the cannabinoid CB_1 receptor antagonist, SR 141716A. Specifically, the drug reduced the number of errors committed following a 6-h delay when given 20 min before the acquisition phase, but not when injected either immediately following the acquisition phase or 20 min before the test phase. These findings indicate that the facilitation was related to the temporal relationship between the drug and the acquisition, not merely a time-dependent effect of the drug. Moreover, these results are consistent with the notion that SR 141716A improved choice accuracy by facilitating attention and/or consolidation, but not retrieval processes.

The possibility that a drug-induced improvement in performance is not simply an effect on cognition, but is the result of changes in motivation or locomotor effects is always a consideration. However, SR 141716A has been reported to decrease feeding and body weight (Colombo et al., 1998), as well as selectively decrease sucrose consumption (Arnone et al., 1997), suggesting that the drug would not increase the salience of the food pellet. In addition, SR 141716A failed to affect the rate of entry into each arm and the subjects always consumed the food pellet regardless of drug condition. Finally, the observation that SR 141716A only enhanced performance during testing when given prior to the acquisition, and not immediately following acquisition or prior to testing, fail to support these alternative explanations.

The apparent memory improving effects of SR 141716A in the delay version of the radial-arm maze task are consistent with the findings from another report in which it enhanced memory as assessed in a social recognition test (Terranova et al., 1996). In this task, however, SR 141716A effectively enhanced performance when given within the first 5 min after the first phase, had no effect when given 15 or 90 min after the first phase, and was not evaluated prior to phase 1 testing. Their results suggest that SR 141716A acted upon consolidation processes and not attentional or retrieval processes. Though SR 141716A could have facilitated performance by enhancing both attention and consolidation in the present study, its effects on retrieval can be ruled out. This discrepancy between the two studies may be related to methodological variables, including the different tasks employed, strain differences (e.g. Terranova et al., 1996 used Wistar rats, while the present study used Long Evans rats), and route of administration (e.g. Terranova et al., 1996 injected the drug subcutaneously, while the present study used the i.p. route). Regardless of this apparent incongruity, both studies taken together demonstrate that SR 141716A can enhance memory.

In contrast to the apparent memory enhancing effects of SR 141716 in the social recognition and radial-arm maze tasks, the drug failed to enhance performance in a variety of operant tasks (Brodkin and Moerschbaecher, 1997; Mallet and Beninger, 1998; Mansbach et al., 1996). The apparent task-dependent effectiveness of SR 141716A may be accounted by the different temporal components of the tasks. In both social recognition and radial-arm maze tasks, the subjects are required to retain the information for substantially longer periods of time than in the operant tasks (i.e. minutes or hours vs. seconds).

The findings of the present study are consistent with the hypothesis that SR 141716A produced its effects by antagonizing a tonically active endogenous cannabinoid system. Alternatively, SR 141716A-induced memory enhancement may be mediated through either the drug's inverse agonist action or noncannabinoid sites of action. Whereas cannabinoid agonists stimulate G-protein activity, SR 141716A has been demonstrated to inhibit G-protein activity, suggesting that SR 141716A has inverse agonist activity at the cannabinoid CB_1 receptor, (Bouaboula et al., 1997; Hillard et al., 1999; Landsman et al., 1997; MacLennan et al., 1998). However, each of these studies used an in vitro preparation in which the cannabinoid CB_1 receptor was over-expressed. On the other hand, other converging evidence implicating the tonic involvement of endogenous cannabinoids in memory is that cannabinoid CB_1 receptor knock-out mice exhibited significantly better memory as assessed in a two-trial object recognition test than the wild-type control mice (Reibaud et al., 1999). Similarly, greater long-term potentiation, an in vitro model of learning and memory, was found in the cannabinoid CB_1 receptor knock-out mice than in the wild-type controls (Bohme et al., 2000).

In conclusion, the results of the present study contribute further evidence that SR 141716A can enhance memory in healthy rats. Moreover, these findings underscore the importance of understanding the involvement of the endogenous cannabinoid system in learning and memory. Finally, SR 141716A may be of benefit in enhancing cognition in pathophysiological states marked by memory deficits, as in

the case of traumatic brain injury or neurodegenerative diseases, such as Alzheimer's disease.

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