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SR141716A, a cannabinoid CB_1 receptor antagonist, improves memory in a delayed radial maze task

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Abstract

An endogenous cannabinoid system may play an important role in controlling memory processes. SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride), a selective cannabinoid CB_1 receptor antagonist, was studied in an eight-arm radial maze task in which either deficits or improvements in memory could be detected. This task required well-trained rats to recall after either a relatively short (3 h) or long (7 h) delay period where they had received rewards during an information phase in order to obtain the remaining rewards during a retention phase. SR141716A was administered intraperitoneally immediately after the information phase in order to determine the drug's effects on memory consolidation. Although SR141716A had no effect on the number of errors committed after a short interval, SR141716A significantly reduced the number of errors that occurred after 7 h. These results suggest that a cannabinoid CB_1 receptor antagonist can improve consolidation processes and thus may be useful in treating memory disorders.

Keywords: Cannabinoid CB_1 receptor antagonist; Memory consolidation; Radial arm maze

1. Introduction

Several lines of evidence suggest that an endogenous cannabinoid system may play an important role in modulating learning and memory. For example, one type of cannabinoid receptor, the CB_1 subtype (Herkenham et al., 1991), and the endogenous ligand, anandamide (Felder et al., 1996), are found in the hippocampus. Also, cannabinoid CB_1 receptor knock-out mice perform significantly better in a two-trial object recognition test than wild-type controls (Reibaud et al., 1999), and exhibit enhanced long-term potentiation (Bohme et al., 2000) in the hippocampus. In contrast, agonists for the cannabinoid CB_1 receptor, such as Δ^9-tetrahydrocannabinol, impair memory in several species (Varvel et al., 2001; Lichtman et al., 1995), whereas SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride), a specific antagonist of the cannabinoid CB_1 receptor (Rinaldi-Carmona et al., 1994), blocked the disruptive effects of cannabinoids on measures of learning and memory (e.g. Brodkin and Moerschbaecher, 1997). However, the cannabinoid CB_1 receptor antagonist, SR141716A, has produced a confusing profile in cognition when administered alone to animals. SR141716A has been reported to impair (Nakamura-Palacios et al., 2000), to have no effect on (Brodkin and Moerschbaecher, 1997, Mansbach et al., 1996), or to improve cognition (Terranova et al., 1996; Lichtman, 2000).

The purpose of the present study was to determine the effect of SR141716A on memory retention in a delayed non-match to sample task conducted in an eight-arm radial maze. Well-trained rats were required to recall where they received food during the information phase in order to obtain the remaining rewards during the retention phase conducted after a delay period. Performance of this task is dependent upon the length of time the information must be retained and by the administration of putative amnesics and cognitive enhancers (Staubli et al., 1994; Pussinen and Sirvio, 1999; Wolff and Leander, 2003). Because relatively few errors occur following a 3-h delay between phases, whereas significantly more errors occur following a 7-

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h delay (Wolff and Leander, 2003), both drug-induced decrements and improvements in memory can be examined using the different delay conditions. A range of doses of SR141716A was administered immediately after the information phase to determine if the compound influenced consolidation processes.

2. Materials and methods

2.1. Subjects

Twenty-one adult (434 ± 10 g), male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were individually housed and maintained on a 12-h light–dark cycle (lights on at 6 a.m.). All testing was conducted at the same time each day during the light phase of the cycle. The rats were given free access to water and were maintained at 85% of their free-feeding weight by supplemental feedings of Lab Diet #5001 for rodents (PMI Nutrition International, St. Louis, MO). This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and the Eli Lilly animal care and use guidelines.

2.2. Drugs

SR141716A was dissolved in 15% cyclodextrin with a drop of TWEEN80. All injections were given intraperitoneally (i.p.) in a volume of 1 ml/kg. SR141716A was supplied by Eli Lilly and Co. (Indianapolis, IN).

2.3. Apparatus and training

The apparatus consisted of an eight-arm radial maze (Habitest, Coulbourn Instruments, Allentown, PA) operated by computer (L2T2S control software, Coulbourn Instruments). Hoppers placed at the end of each arm delivered a single food reward (45 mg pellet, BioServ, Frenchtown, NJ) at the beginning of each session. In order to count as an arm entry, the rat was required to run all of the way to the end of the alley and to cross a photo-beam located at the opening of the food magazine.

All rats were initially trained to search for food at the end of each of the eight arms. Once the rats had reached the criteria of no more than two errors (i.e. entering the same arm more than once during a session) on 3 consecutive days, delay training was begun using a 1-min delay between the information and retention phases. During the information phase, the rat was placed on the central platform with access to all of the alleys blocked. The gates to 4, randomly selected, baited arms were raised. The information phase lasted until all four alleys had been entered or until 5 min elapsed. The rat was removed from the last alley entered and placed back on the center platform. One minute later, the retention phase began. The four previously blocked alleys were baited, and all eight of the guillotine doors were raised. The rat was allowed to freely explore the maze until the four remaining pieces of food had been retrieved or until 5 min elapsed. Only the initial visit to a baited arm was rewarded each day. During the retention phase, any entry into an arm that had been baited during the information phase or re-entry into one of the arms baited during the retention phase was counted as an error. Total errors also included any errors of omission (failures to enter a baited arm). Once stable performance had been obtained (i.e. no more than one error was made on 3 consecutive days), testing commenced. Either a relatively short, 3-h delay (seven rats) or a relatively long, 7-h delay (eight rats) between the information and retention phases was used. At the conclusion of the information phase, the rat was removed from the maze, injected i.p. with vehicle or SR141716A, and placed back in its home cage for the delay period. Each rat was tested first with vehicle and then once, in a semi-random order, with all doses of SR141716A. After all doses of SR141716A had been completed, each rat was tested with vehicle once more.

A novel set of arms was baited each day for each rat and the maze was thoroughly cleaned with a 70% isopropyl alcohol solution during the delay period. Drug or vehicle tests were conducted on Tuesday and Friday. The animals were not tested on the intervening days.

In order to control for the possibility SR141716A also affected retrieval processes, six additional rats were tested with the pre-determined effective dose of SR141716A (1 mg/kg). This experiment was conducted in exactly the same manner as above, except that either vehicle or drug was injected 1 h prior to the retention phase conducted after a delay of 7 h.

2.4. Statistics

The data are expressed as the mean number of errors that occurred during the retention phase (± S.E.M.). Statistical significance was determined using a repeated measure analysis of variance (ANOVA) followed by a Dunnett's test for comparison with control (GraphPad Prism 3.02) or by a paired *t*-test in the case of the retrieval test. The latency and the total time taken to complete the maze during the information and retention phases were also recorded and analyzed in the same manner as the errors. The vehicle control data of the two groups (3- and 7-h delay) were compared by *t*-test to ensure that the tests were comparable in all respects except the number of errors in the retention phase.

In order to ensure that there was a baseline of performance from which a potential deficit could be observed in the 3-h delay condition, rats were required to maintain a consistent performance of no more than one error during the retention phase during both vehicle control sessions. Application of these criteria did not result in the loss of any data

in the 3-h delay condition. It has been our experience that, if rats are continuously tested at any one given delay between the information and retention phases, some will learn to do the task without error, even in the absence of drug after a relatively long delay between phases. To prevent such a shift in baseline from biasing the results, each rat's performance under drug was always compared to its own baseline obtained both before and after the period of drug administration. If a rat made fewer than two errors during either of the vehicle control sessions in the 7-h delay condition, this was taken as evidence that the decrease in error under drug could have been due to processes unrelated to a specific drug effect. Thus, all data from those rats were eliminated from the analysis. Application of these criteria resulted in the loss of data from two rats in the 7-h delay tests. These were very rigorous criteria that were applied in order to be conservative about interpreting an effect as being a specific drug effect.

3. Results

After a 3-h delay, rats made 0.5 ± 0.2 errors during the retention phase, whereas after the 7-h delay, rats made 4.2 ± 0.6 errors ($P < 0.0001$). There were no significant differences in the latency to start (5.7 ± 1.1 and 6.6 ± 1.9 s) or complete (111 ± 27 and 112.3 ± 28 s) the information phases for the 3- and 7-h delay conditions, respectively. Likewise, there were no significant differences in the latency to start (4.4 ± 3 and 5.6 ± 2 s) or to complete (103 ± 9 and 112 ± 16 s) the retention phases. During the determination of the dose response data, there were no significant differences in error, latency or time to complete the information phases in either the 3- or the 7-h delay conditions.

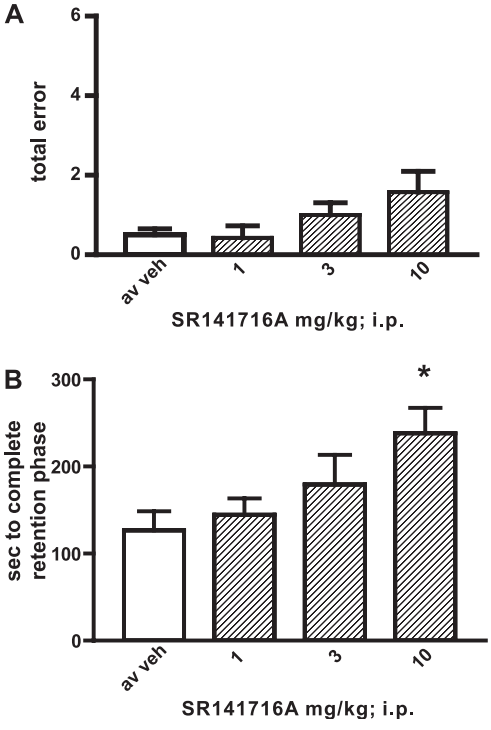

Fig. 1. (A) The average number of errors that occurred during the retention phase conducted after a delay of 3 h. Doses of SR141716A were administered i.p. immediately after the information phase. Bars indicate the S.E.M.; $N = 7$. (B) The time required in order to complete the retention phase after a 3-h delay in the same rats.

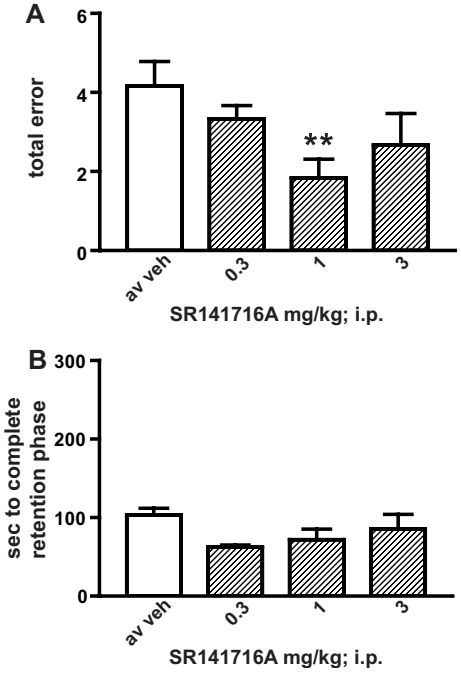

Fig. 2. (A) The average number of errors that occurred during the retention phase conducted after a delay of 7 h. Doses of SR141716A were administered i.p. immediately after the information phase. Bars indicate the S.E.M.; $N = 6$. (B) The time required in order to complete the retention phase after a 7-h delay in the same rats.

Even relatively high doses (3 and 10 mg/kg) of SR141716A had no effect on the total number of errors committed after a 3-h delay (Fig. 1A). However, there was a significant increase in the number of errors of omission (0 after vehicle to 0.86 (± 0.4) after 10 mg/kg; $F_{(3,18)} = 3.13$; $P = 0.05$). The time required to complete the retention phase ($F_{(3,18)} = 4.45$; $P = 0.02$) also increased significantly (Fig. 1B).

If injected immediately after the information phase, SR141716A (1 mg/kg, i.p.) decreased the number of errors that occurred during the retention phase ($F_{(3,15)} = 4.9$; $P = 0.02$) conducted 7 h later (Fig. 2A). After a 7-h delay, no errors of omission occurred after either vehicle or drug injections. Neither the latency to start the retention phase nor the length of time required to complete the retention phase was affected by the injection of SR141716A (Fig. 2B). It is noteworthy that at a higher dose (3 mg/kg) the memory enhancing effect of SR141716A was lost.

When administered 1 h prior to the retention session, SR141716A (1 mg/kg, i.p.) had no effect on the number of

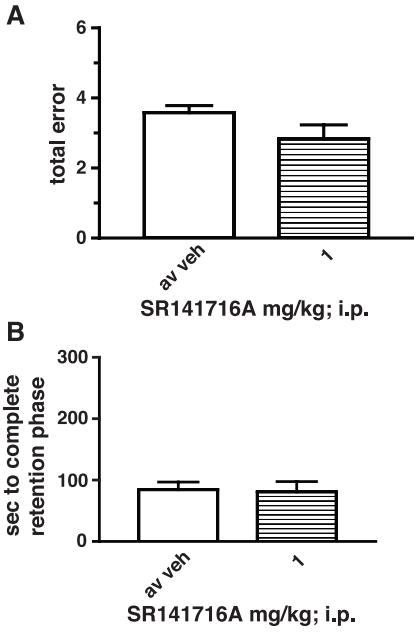

Fig. 3. (A) The average number of errors that occurred during the retention phase conducted after a delay of 7 h. SR141716A was administered i.p. 1 h prior to the retention session. Bars indicate the S.E.M.; $N=6$. (B) The time required in order to complete the retention phase after a 7-h delay in the same rats.

errors (Fig. 3A). Likewise, there was no significant effect upon the time required to complete the task (Fig. 3B).

4. Discussion

Increasing the delay period between the information and retention phases from 3 to 7 h resulted in a significant increase in the number of errors committed during the retention phase. Thus, it was possible to determine whether SR141716A increased or decreased errors in tests that were comparable in all respects, with the exception of the number of errors generated during the retention phase.

With a 7-h delay between the information and retention phases of the radial maze test, 1 mg/kg (but not higher or lower doses) of SR141716A decreased errors during the retention phase. Because the drug was not injected until immediately after the information phase, SR141716A most likely enhanced consolidation processes, rather than acquisition or retrieval processes. This inference is supported by the fact that when injection of SR141716A was delayed until 1 h prior to the retention phase of the 7-h delay condition, the number of errors did not decrease. In agreement with these results, Terranova et al. (1996) found that recognition of a novel juvenile rat by an adult rat was improved if 1 mg/kg of SR141716A was administered immediately after the original encounter, but not if injected just prior to the recall session. Lichtman (2000) found that 3 mg/kg of SR141716A did not improve retention when administered after the acquisition phase of a radial arm maze task, but was effective if administered before the acquisition phase. Unfortunately, lower doses of SR141716A were not administered in that study (Lichtman, 2000). The apparent differences in results between these studies might be explained by the narrow dose range over which SR141716A appears to improve cognitive performance.

Because of the limited dose range over which SR141716A was effective, and because others reported impairment (Nakamura-Palacios et al., 2000) following administration of SR141716A, the drug was tested using a much shorter delay (3 h) between information and retention phases, a situation in which very few errors normally occur and which allows testing for potential cognitive impairment. In the 3-h delay condition, the rats' ability to complete the maze was compromised at 10 mg/kg as indicated by the increased time to complete the maze and the increased errors of omission. However, at this dose, the arm choices that were made remained accurate. The deficit in responding at 10 mg/kg may be due to unrelated effects of SR141716A, such as its tendency to reduce food intake or decrease motivation (for review, see Chaperon and Thiebot, 1999). The finding of reduced responding in the present experiment complements findings in operant behavior tests (Mansbach et al., 1996), where high doses of SR141716A decreased response rate, but had little effect on task accuracy.

In contrast to the present results, SR141716A did not decrease errors in operant behavior tasks (Brodkin and Moerschbaecher, 1997; Mansbach et al., 1996). This could be due to differences in the behavioral requirements of the tasks or to differences in the sensitivity of the tasks for detecting drug-induced enhancement. It has been suggested that the endogenous cannabinoid system is involved with "spontaneous forgetting" (Terranova et al., 1996), and SR141716A has been found to impede extinction of a fear response (Marsicano et al., 2002). Because the information had to be retained for a much longer period of time when a 7-h delay was interposed in the radial maze task than in the operant tasks, perhaps the radial maze task is more sensitive to any cognitive enhancing effects of the antagonist.

In the present study, SR141716A improved memory in healthy adult rats by apparently enhancing consolidation processes. The dose of SR141716A, a potent selective cannabinoid CB_1 receptor antagonist, that produced this effect was within the reported range of antagonistic activity for this compound (e.g. Brodkin and Moerschbaecher, 1997; Perio et al., 1996). In conjunction with other data demonstrating the importance of an endogenous cannabinoid system in learning and memory processes (see Introduction), these data suggest that a cannabinoid CB_1 receptor antagonist may be useful in treating disorders that involve cognitive deficits.

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