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Aron H. Lichtman · Billy R. Martin

Δ^9-Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism

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Abstract The purpose of the present study was to investigate whether the cannabinoid and cholinergic systems impair working memory through a common mechanism. This hypothesis was tested by examining whether the cannabinoid antagonist SR141716A would ameliorate radial-arm performance deficits caused by either the naturally occurring cannabinoid, Δ^9-THC, or scopolamine, a muscarinic antagonist. In addition, we evaluated whether the cholinesterase inhibitor, physostigmine, would prevent Δ^9-THC-induced impairment of spatial memory. Finally, because the locomotor suppressive effects of cannabinoids may decrease radial arm choice accuracy independent of a direct effect on memory, we examined the impact of increasing the intertrial error on radial arm choice accuracy. As previously reported, Δ^9-THC impaired maze performance (ED_{50}=3.0 mg/kg). Increasing the intertrial interval from 5 s to 30 s resulted in a threefold increase in the amount of time required to complete the maze without affecting choice accuracy. Importantly, SR141716A prevented Δ^9-THC-induced deficits in radial-arm choice accuracy in a dose-dependent manner (AD_{50}=2.4 mg/kg); however, the cannabinoid antagonist failed to improve the disruptive effects of scopolamine. Conversely, physostigmine failed to improve performance deficits produced by Δ^9-THC. These data provide strong evidence that Δ^9-THC impairs working memory through direct action at cannabinoid receptors. Moreover, these results suggest that scopolamine and Δ^9-THC do not impair spatial memory in a common serial pathway, though they may converge on a third neurochemical system.

Key words Radial-arm maze · Δ^9-Tetrahydrocannabinol (Δ^9-THC) · Scopolamine · Physostigmine · SR141716A · Cannabinoid antagonist · Working memory

A.H. Lichtman (✉) · B.R. Martin
Department of Pharmacology and Toxicology, P.O. Box 980613, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298, USA

Introduction

The naturally occurring and synthetic cannabinoids produce many pharmacological effects in in vivo and in vitro systems (Dewey 1986; Martin 1986). The deficits in cognitive functioning (Abel 1971) associated with marijuana use in humans is of considerable concern. An active constituent of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), and other cannabinoids have been demonstrated to impair learning and memory in primates as well as rodents (Carlini et al. 1970; Ferraro and Grilly 1973; Evans and Wenger 1992; Heyser et al. 1993). These compounds also disrupt working memory in the eight arm radial-maze task (Nakamura et al. 1991; Lichtman et al. 1995).

The existence of a structure activity relationship as well as neuroanatomical evidence suggests that cannabinoids impair working memory through a cannabinoid-receptor mechanism. The potent bicyclic analog, CP55,940, was approximately 16-fold more potent than both Δ^9-THC and the aminoalkylindole compound WIN55,212–2 in disrupting memory (Lichtman et al. 1995). In addition, cannabidiol, an inactive naturally occurring cannabinoid, failed to alter maze performance at doses up to 30 mg/kg. The relative potencies of these drugs in impairing memory in the radial-arm maze are similar to their potencies in eliciting other cannabinoid pharmacological effects and relative affinity to the cannabinoid receptor (Little et al. 1988; Compton et al. 1992a,b, 1993). Other evidence consistent with a cannabinoid receptor mechanism is that intracerebral administration of CP55,940 into the hippocampus, a brain structure implicated in learning and memory and containing a high density of cannabinoid receptors, disrupted working memory in the radial-arm maze task (Lichtman et al. 1995).

Though the above findings are consistent with a receptor mechanism of action, the reversal of cannabinoid-induced memory disturbances by a cannabinoid antagonist would provide more compelling support for such a hypothesis. The discovery of the cannabinoid antagonist

SR141716A provides a useful tool to discern whether a pharmacological effect is mediated through a cannabinoid receptor mechanism. SR141716A binds to CB1, but not CB2, receptors and prevents the inhibitory effects of cannabinoids on adenylyl cyclase accumulation, cerebellar cGMP and twitch contractions of the mouse vas deferens (Rinaldi-Carmona et al. 1994, 1995). Of importance, the inhibitory effects of the potent cannabinoid HU-210 on long-term potentiation in the rat hippocampal slice, a neural model for learning and memory, are blocked by SR141716A (Collins et al. 1995). This antagonist also blocks a variety of in vivo responses, including antinociceptive, cataleptic, hypothermic, and other motor effects of cannabinoids (Rinaldi-Carmona et al. 1994, 1995). In addition, SR141716A antagonizes the discriminative stimulus cues of Δ^9-THC, CP55,940, and WIN55,212–2 in the drug discrimination paradigm in rats (Wiley et al. 1995a). The primary goal of the present study, therefore, was to evaluate whether cannabinoid receptors mediate Δ^9-THC-induced memory impairment in the radial-arm maze using SR141716A.

The integrity of central cholinergic pathways has been demonstrated to play a critical role for memory as assessed in the radial-arm maze. Administration of scopolamine, a muscarinic antagonist, or destruction of central cholinergic neurons in the forebrain has been demonstrated to impair working memory in the radial-arm maze (Olton 1987). Importantly, these memory deficits can be reversed with physostigmine and other cholinergic agonists (Murray and Fibiger 1985; Tilson et al. 1988; Matsuoka et al. 1991; McGurk et al. 1991; Dennes and Barnes 1993). Cannabinoids increase acetylcholine in a variety of brain regions including the hippocampus (Revuelta et al. 1980; Tripathi et al. 1987); however, they have also been shown to reduce acetylcholine release in the periphery (Paton and Aboo Zar 1968; McConnell et al. 1978). In addition, there is some indication of a functional interaction between cannabinoid and cholinergic systems in catalepsy (Ferri et al. 1981; Pertwee and Ross 1991; Prescott et al. 1992). The presence of cholinergic and cannabinoid receptors in the hippocampus and other brain areas associated with memory suggests the possibility that these two systems may modulate working memory through a common serial pathway.

The second objective of the present study, therefore, was to evaluate whether cannabinoid and cholinergic systems affect memory through a common serial pathway. Two approaches were taken to accomplish this aim. First, the effects of SR141716A pretreatment on scopolamine-induced memory impairment were evaluated in the radial-arm maze. Any improvement in choice accuracy resulting from SR141716A pretreatment would suggest the functional involvement of endogenous cannabinoids; or, conversely, that the cannabinoid antagonist is nonselective. Second, the involvement of cholinergic systems in Δ^9-THC-induced impairment of radial-arm maze choice accuracy was assessed by pretreating rats with physostigmine. As a cholinesterase inhibitor, physostigmine acts as a nonspecific agonist at nicotinic as well as muscarinic receptors. If cannabinoids impair working memory by inhibiting cholinergic transmission, physostigmine would be predicted to improve radial-arm maze performance in animals administered Δ^9-THC.

Finally, in addition to impairing choice accuracy in the radial-arm maze, cannabinoids are known to inhibit locomotor activity (Dewey 1986; Martin 1986). This raises the possibility that these drugs do not impair radial-arm maze performance by a direct effect on memory, but merely increase the difficulty of the task by retarding the animal's latency to enter an arm. The third objective of this study, therefore, was to evaluate whether the inhibitory effects of Δ^9-THC on locomotor activity contribute to the decrements in choice accuracy. This hypothesis was tested by examining whether increasing the intertrial-interval (ITI) from 5 s to 30 s would impair choice accuracy in either vehicle- or Δ^9-THC-treated rats.

Materials and methods

Subjects

Two groups of Sprague-Dawley (Harlan, Ind.) male rats served as subjects (n=12 for each group). The animals 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 and maintained at a weight between 290 and 300 g, approximately 85% of their free-feeding weight, for the duration of the experiment. Daily food rations (12–16 g, Prolab, Agway) were given immediately after each experimental session, and water was available ad libitum at all times except during the test session.

Drug preparation and administration

Δ^9-THC was provided by the National Institute on Drug Abuse and SR141716A was provided by Pfizer (Groton, Conn.). Scopolamine and physostigmine were purchased from Sigma (St Louis). The cannabinoids were dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (formerly called emulphor-620; Rhone-Poulenc, Princeton, N.J.), and diluted with saline. The vehicle ratios for Δ^9-THC and SR141716A were 1:1:18 (ethanol:alkamuls:saline) and 1:1:8 (ethanol:alkamuls:saline), respectively. Saline served as the vehicle for scopolamine and physostigmine. All injections were given through the IP route of administration in a volume of 1 ml/kg.

Apparatus and training

The equipment and training procedure were identical to that described elsewhere (Lichtman et al. 1995). Each of the eight arms was baited with a 45-mg Noyes pellet placed 5 cm from the end. At the start of a session, the subject was placed on the center platform with all doors down. Five seconds later, all of the doors were raised. Subjects were defined as selecting an arm once it crossed the threshold into a maze arm. Once a selection was made, the other seven guillotine doors were lowered. After the subject returned to the center platform, the remaining door was lowered for the 5-s ITI; all eight doors were then raised for the next trial. The session ended when either each arm was visited or 10 min had elapsed, whichever occurred first. The experimenter recorded which arm was visited and whether the pellet was consumed for each entry, as well as the duration of the session. Subjects became proficient in the task within approximately 20 training sessions.

Behavioral testing

Subjects were evaluated in the maze 20 min after the injection of Δ^9-THC (0, 2, 4, or 6 mg/kg) or scopolamine (0, 0.1, or 0.5 mg/kg). A dose of 5 mg/kg Δ^9-THC was used in the antagonism studies in an attempt to produce significant decreases on radial-arm maze choice accuracy while limiting the sedative effects of the drug. SR141716A (0, 1, 3, or 10 mg/kg) was given 10 min before vehicle or Δ^9-THC. SR141716A (0, 3, or 10 mg/kg) was also given 10 min before each of the scopolamine doses. Physostigmine (0, 0.06, 0.12, or 0.24 mg/kg) was administered 10 min before vehicle or Δ^9-THC. These doses of physostigmine have been previously reported to reverse deficits in maze performance caused by neurochemical lesions to cholinergic neurons in the CNS (Tilson et al. 1988). In the experiment assessing the impact of a delay in the ITI, subjects received either vehicle or the ED50 dose of Δ^9-THC (3 mg/kg) and the ITIs consisted of either 5 or 30 s.

The first group of subjects was used in the Δ^9-THC dose-response study, and seven of these rats were consequently used in the ITI delay experiment. The second group of rats was used in each of the other experiments. The order of experiments for these subjects was: 1) SR141716A versus Δ^9-THC, 2) SR141716A versus scopolamine, and 3) physostigmine versus Δ^9-THC. For each experiment, the dose regimens were counter-balanced among subjects to control for any order effects. Each subject was given no more than one injection per week after successfully completing the maze on 2 consecutive days. As subjects became more proficient in the task, they were given one training session per week prior to the test day.

Statistical analyses

The four dependent measures of interest were the number of reentries, the trial at which the first error occurred, the total amount of time required to complete the maze, and the number of correct entries. The number of entries before the first error occurred was determined by the following formula: 8–number of arms remaining unvisited when the first error occurred. Accordingly, an 8 indicated that no errors were committed, a 7 indicated that the error was committed on the eighth selection, and a 1 meant that the first error was made on the second choice. The two variables indicative of choice accuracy, the number of reentries and the trial at which the first error occurred, were used to infer whether the Δ^9-THC impaired spatial memory. A one-way ANOVA was used to analyze each dependent measure in the dose response Δ^9-THC experiment, the SR141716A versus Δ^9-THC experiment, and the physostigmine versus Δ^9-THC experiment. Two-way ANOVA was used in the SR141716A versus Δ^9-THC experiment and the time delay experiment. The Newman-Keuls test was used for post-hoc analysis. Differences were considered significant at the $P<0.05$ level.

The potency of Δ^9-THC in disrupting radial-maze performance was calculated by dividing the mean number of errors for each drug condition by the mean number of errors committed in the high-dose condition. In order to calculate the potency of SR141716A in antagonizing the disruptive effects of Δ^9-THC on maze performance, the data for each dose of SR141716A were transformed to percent antagonism by the following formula: 100×(number of errors in vehicle-Δ^9-THC condition minus number of errors in SR141716A-Δ^9-THC condition)/(number of errors in vehicle-Δ^9-THC condition). The ED_{50} value of Δ^9-THC and the AD50 value of SR141716A were then calculated (Tallarida and Murray 1987).

Results

Dose-related effects of Δ^9-THC on spatial memory

The ED_{50} dose of Δ^9-THC in impairing choice accuracy was 3.0 mg/kg. The number of arms revisited was significantly increased by Δ^9-THC [$F(3, 33)=7.7$, $P<0.05$; Fig. 1A]. Subjects committed significantly more errors when treated with 6 mg/kg Δ^9-THC than each of the other conditions ($P<0.05$). The drug also reduced the number of arms visited before the first error was committed [$F(3, 33)=4.8$, $P<0.05$; Fig. 1B]. The first reentry occurred significantly earlier with the 6 mg/kg dose than with vehicle or 2 mg/kg drug, and the 4 mg/kg dose significantly differed from the vehicle condition ($P<0.05$). Δ^9-THC significantly increased the amount of time required to complete the task [$F(3, 33)=7.2$, $P<0.05$; see Fig. 1C]. Subjects required significantly more time to complete the maze after treatment with 6 mg/kg drug than each of the other conditions ($P<0.05$). Finally, Δ^9-THC had no impact on the number of correct entries made [$F(3, 33)=0.9$, $P>0.40$; see Fig. 1D].

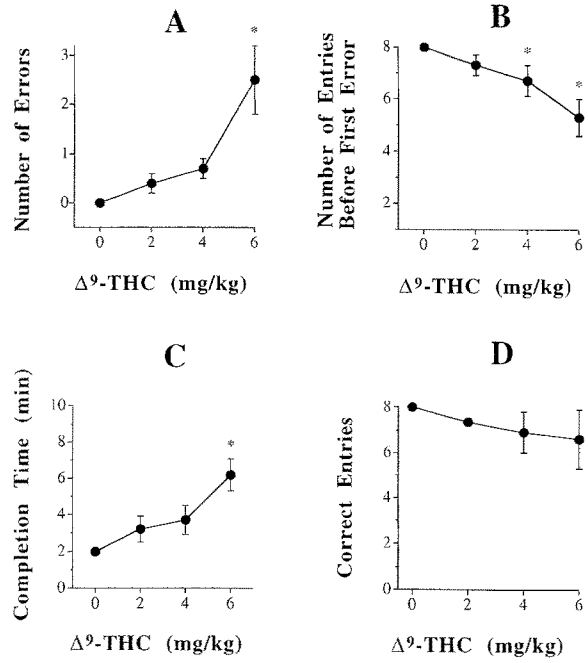

Fig. 1A–D The effects of IP Δ^9-THC on eight arm radial-maze performance. All results are presented as means±SEM (*significantly different from vehicle). **A** The number of errors committed during testing. **B** The trial at which the first error occurred. **C** The amount of time required to complete the maze. **D** The total number of correct entries made for each condition

Effects of SR141716A pretreatment on Δ^9-THC-induced memory impairment

As can be seen in Fig. 2, the cannabinoid antagonist, SR141716A, antagonized the disruptive effects of Δ^9-THC (5 mg/kg) on spatial memory in a dose-dependent fashion (AD_{50} dose=2.4 mg/kg). SR141716A completely blocked the disruptive effects of Δ^9-THC in the radial-arm maze as indicated by the return to baseline levels (Fig. 3). Significant effects were found for both dependent measures indicative of choice accuracy [$F(6, 113)=6.8$, $P<0.05$ for the number of reentries committed

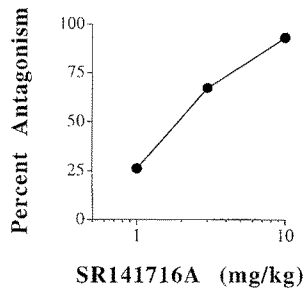

Fig. 2 The antagonistic effects of IP SR141716A on Δ^9-THC-induced impairment on spatial memory (AD_{50} value= 2.4 mg/kg). Subjects were administered SR141716A 10 min prior to Δ^9-THC (5 mg/kg) and tested in the radial-arm maze at 30 min

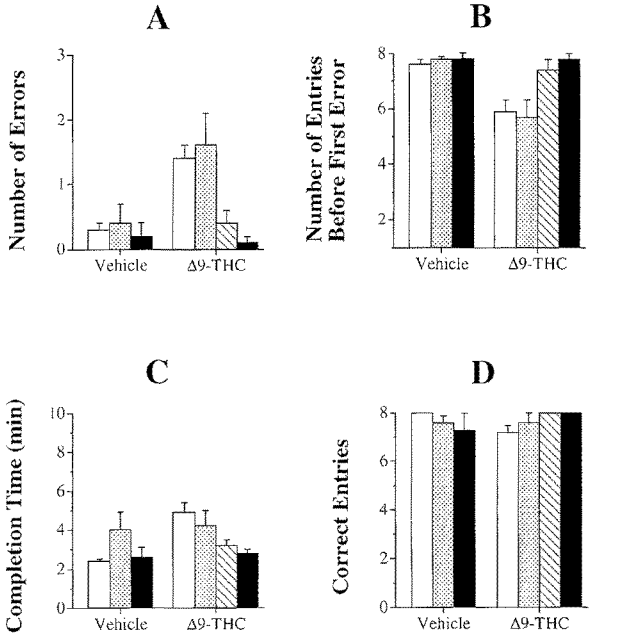

Fig. 3A–D The effects of pretreatment of vehicle (□), or 1 (▨), 3 (▧), or 10 mg/kg (■) of SR141716A prior to vehicle or 5 mg/kg Δ^9-THC on eight arm radial-maze performance. All results are presented as means±SEM (see text for statistics). **A** The number of errors committed during testing. **B** The trial at which the first error occurred. **C** The amount of time required to complete the maze. **D** The total number of correct entries made for each condition

(Fig. 3A) and $F(6, 113)=7.1$, $P<0.05$ for the trial at which the first error occurred (Fig. 3B)]. Subjects pretreated with either vehicle or 1 mg/kg SR141716A prior to Δ^9-THC exhibited significant decrements in both measures of choice accuracy compared to each of the other groups. A significant effect was also found for the amount of time required to complete the maze [$F(6, 113)=4.8$, $P<0.05$ (Fig. 3C)]. The Δ^9-THC-injected rats that were pretreated with the vehicle required significantly more time to complete the maze than the vehicle-vehicle, 10 mg/kg SR141716A-vehicle, and the 10 mg/kg SR141716A-Δ^9-THC conditions. In contrast, none of the drug conditions affected the total number of correct entries choices [$F(6, 113)=1.2$, $P>0.25$; Fig. 3D).

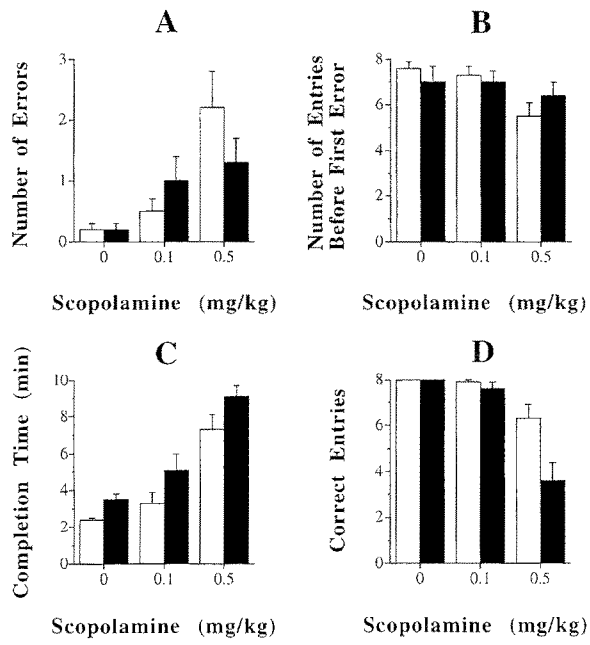

Fig. 4A–D The effects of pretreatment of vehicle (□) or 10 mg/kg SR141716A (■) prior to vehicle or scopolamine on eight-arm radial-maze performance. All results are presented as means±SEM (see text for statistics). **A** The number of errors committed during testing. **B** The trial at which the first error occurred. **C** The amount of time required to complete the maze. **D** The total number of correct entries made for each condition

Effects of SR141716A pretreatment on scopolamine-induced memory impairment

Scopolamine impaired both dependent measures indicative of choice accuracy [$F(2, 22)=11.7$, $P<0.05$ for the total number of reentries (Fig. 4A) and $F(2, 22)=4.7$, $P<0.05$ for the trial at which the first error occurred (Fig. 4B)]. For both variables, the 0.5 mg/kg dose of scopolamine decreased choice accuracy more than the other conditions and performance was disrupted with 0.1 mg/kg of scopolamine compared to the vehicle ($P<0.05$). Significant main effects were found for both scopolamine [$F(2, 22)=30.2$, $P<0.05$] and SR141716A [$F(1, 11)=14.2$, $P<0.05$] on maze completion time (Fig. 4C). Similarly, there were significant main effects for scopolamine [$F(2, 22)=17.0$, $P<0.05$] as well as SR141716A [$F(1, 11)=32.2$, $P<0.05$] on the number of correct entries made (Fig. 4D). No significant interaction was found between scopolamine and SR141716A for any of the dependent variables. Although subjects treated with SR141716A and the 0.5 mg/kg dose of scopolamine committed some errors of omission, these subjects did not appear sedated or immobile; much of the testing period was spent locomoting in the central platform. Nonetheless, they visited most of the arms and committed significantly more errors of reentry than the vehicle-vehicle group.

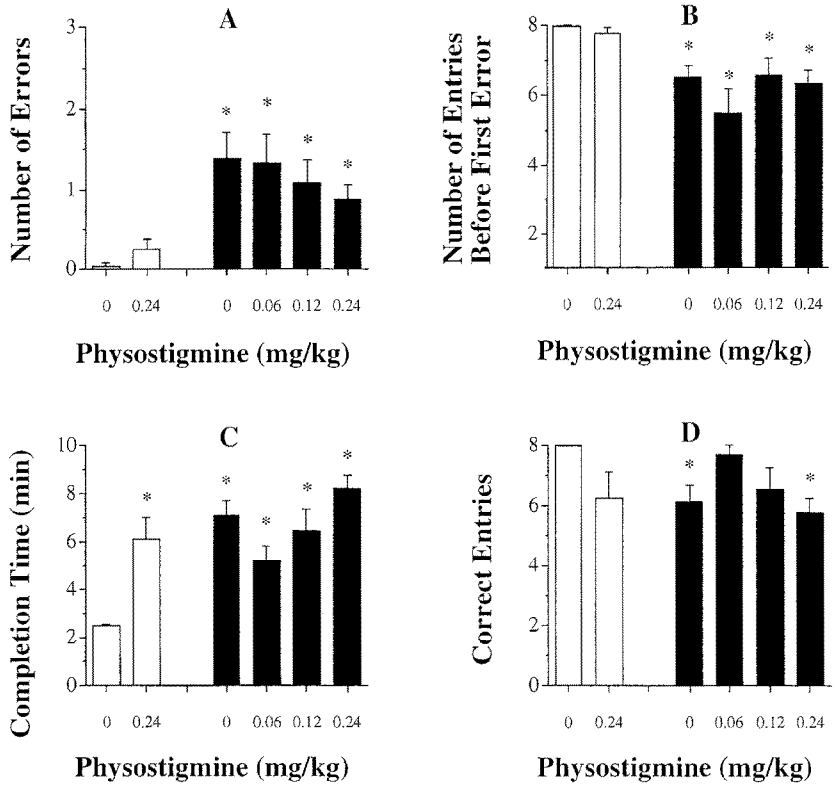

Fig. 5A–D The effects of physostigmine pretreatment prior to vehicle (□) or 5 mg/kg Δ^9-THC (■) on eight arm radial-maze performance. All results are presented as means±SEM (*significantly different from the vehicle-vehicle condition). **A** The number of errors committed during testing. **B** The trial at which the first error occurred. **C** The amount of time required to complete the maze. **D** The total number of correct entries made for each condition

Table 1 The effects of imposing a delay in the ITI on radial-arm maze performance (all data are expressed as mean±SEM, see text for statistics)

Condition	ITI (s)	Number of errors	Number of entries before first error	Completion time (min)	Number of correct entries
Vehicle	5	0.0±0.0	8.0±0.0	2.3±0.2	8.0±0.0
	30	0.4±0.4	7.1±0.4	6.2±0.4	8.0±0.0
Δ^9-THC	5	0.7±0.2	7.1±0.4	3.0±0.4	8.0±0.0
	30	1.4±0.5	5.7±0.9	8.2±0.7	7.9±0.2

Effects of physostigmine pretreatment on Δ^9-THC-induced memory impairment

Figure 5 depicts the effects of pretreatment with either vehicle or physostigmine (0.06, 0.12, or 0.24 mg/kg) prior to Δ^9-THC (5 mg/kg) in the radial-arm maze. Excessive salivation was observed in subjects treated with the high dose of physostigmine. Significant effects were found for both the number of errors committed in the maze [$F(5, 102)=5.5$, $P<0.05$ (Fig. 5A)] and the number of entries that occurred before the first error [$F(5, 102)=5.6$, $P<0.05$ (Fig. 5B)]. Δ^9-THC significantly impaired choice accuracy regardless of physostigmine pretreatment. A significant effect was also found on the amount of time required to complete the maze [$F(5, 102)=13.9$, $P<0.05$ (Fig. 5C)]. Each drug condition led to a significant increase in maze completion time compared to the vehicle-vehicle treatment. Finally, a significant effect was found for the number of correct arms visited [$F(5, 102)=3.5$, $P<0.05$ (Fig. 5D)], subjects visited significantly fewer arms in the vehicle-Δ^9-THC and the physostigmine (0.24 mg/kg)-Δ^9-THC conditions than the vehicle-vehicle condition.

Effects of increasing the ITI on radial-arm maze performance

The impact of increasing the ITI from 5 s to 30 s is presented in Table 1. Δ^9-THC significantly increased the number of errors committed [$F(1, 6)=10.8$, $P<0.05$), reduced the number of entries before the first error was committed [$F(1, 6)=8.7$, $P<0.05$), and delayed maze completion time [$F(1, 6)=7.9$, $P<0.05$). Although imposing a 30-s delay significantly increased the time required to complete the maze compared to the 5-s ITI [$F(1, 6)=57$, $P<0.05$], it failed to significantly affect either measure indicative of choice accuracy and did not significantly increase the errors of omission ($P>0.1$ for the main effect of delay and the delay by drug interactions for each of these dependent variables).

Discussion

The complete prevention of Δ^9-THC-induced working memory deficits by SR141716A provides strong evidence that cannabinoids impair working memory

through cannabinoid receptors. The failure of physostigmine to improve Δ^9-THC-induced deficits in radial-arm maze performance, however, suggests that cannabinoids do not impair working memory by inhibiting cholinergic transmission. Conversely, endogenous cannabinoids may be under tonic inhibition by acetylcholine. However, the inability of SR141716A to prevent scopolamine-induced decreases in radial maze choice accuracy is not consistent with this second postulate. The failure of SR141716A to prevent the disruptive effects of scopolamine also supports its selectivity to cannabinoid receptors.

The results of the present study suggest that cannabinoid and cholinergic drugs do not impair spatial memory through a common serial pathway. Alternatively, cannabinoid and cholinergic neurons may disrupt radial-arm performance by converging on a third receptor system. One candidate may be dopaminergic neurons; haloperidol and a selective D_1 agonist have alleviated scopolamine-induced deficits in working memory (Levin and Rose 1991). Furthermore, various dopaminergic ligands alleviated the performance deficits caused by lesions to cholinergic fibers that innervate the forebrain (McGurk et al. 1992). Of course, there is the possibility that Δ^9-THC and scopolamine impair spatial memory through entirely separate neurochemical systems.

The increase in maze completion time caused by Δ^9-THC as well as scopolamine suggests that the locomotor suppressive effects of these drugs may have decreased choice accuracy independent of direct drug effects on memory. In other words, the increased amount of time required to complete the maze may have elicited errors merely by increasing the difficulty of the task. Several observations, however, argue against this alternative explanation. Increasing the ITI failed to impair choice accuracy and failed to produce errors of omission, even though it led to a large increase in maze completion time. In addition, the 0.24 mg/kg dose of physostigmine significantly increased maze completion time, but did not affect choice accuracy. Conversely, intrahippocampal administration of CP-55,940, a potent cannabinoid analog, impaired choice accuracy without retarding maze completion time (Lichtman et al. 1995). Similarly, Δ^9-THC and CP-55,940 impaired memory at lower doses than in producing locomotor suppression (Nakamura et al. 1991; Lichtman et al. 1995). The dissociation between radial-arm maze choice accuracy and inhibition of locomotor activity suggests that cannabinoids impair working memory independent of their locomotor effects.

The potency of Δ^9-THC in disrupting radial maze performance found here was similar to the value previously reported (Lichtman et al. 1995). In addition, SR141716A blocked working memory deficits as well as the delay in maze completion time produced by Δ^9-THC. The AD_{50} of the antagonist in blocking cannabinoid-induced antinociception, hypothermia, and other motor effects (Rinaldi-Carmona et al. 1994; Compton et al. 1996), as well as the discriminative cue of cannabinoids (Wiley et al. 1995b), has been reported to range from about 0.1 to 0.8 mg/kg, lower than that in the present study. Although the AD50 dose of SR141716A in the present study appears to be somewhat higher than that required in other in vivo tests, it is similar to the AD_{50} dose in blocking the antinociceptive effects of Δ^9-THC in PBQ-induced writhing (Compton et al. 1996). Thus, it appears that the dose of antagonist required to block the pharmacological actions of cannabinoids may be dependent on the specific effect of interest as well as the dose of agonist employed.

The heterogeneous distribution of cannabinoid receptors (Herkenham et al. 1991) and the presence of endogenous cannabinoids in the CNS (Devane et al. 1992) suggest the existence of a cannabinoid neurochemical system. Although exogenously administered cannabinoids impair cognition, it is unknown whether an endogenous cannabinoid system plays a role in memory processes. Of particular interest is the observation that cannabinoid receptors were decreased in the hippocampi of Alzheimer's patients (Westlake et al. 1994). In rats, however, IP administration of the endogenous cannabinoid anandamide failed to impair working memory as assessed in the radial-arm maze (Lichtman et al. 1995) or the delayed nonmatch-to-sample (Crawley et al. 1993). The rapid metabolism of the drug (Deutsch and Chin 1993) may have prevented it from reaching the necessary sites, however. Alternatively, the use of cannabinoid antagonists, such as SR141716A, may be used to elucidate the function of a putative cannabinoid system in a variety of behaviors, including cognition. The cannabinoid antagonist may also serve to ascertain the involvement of the hippocampus and other brain areas in Δ^9-THC-induced disruption of working memory. Although the high degree of radial-arm choice accuracy exhibited by subjects in the present study precluded the possibility of improving maze performance with SR141716A, future research is likely to focus on whether antagonism of endogenous cannabinoids can improve working memory.

In conclusion, the failure of SR141716A to block the disruptive effects of scopolamine on spatial memory and the failure of physostigmine to block those of Δ^9-THC suggest that cannabinoid and cholinergic agents do not work through a common serial pathway. Nonetheless, it is possible that both drug classes activate a common neurochemical system to disrupt memory. In addition, increasing the ITI from 5 s to 30 s resulted in a three-fold increase in the amount of time required to complete the maze without affecting choice accuracy. Finally, the finding that SR141716A completely blocked the disruptive effects of Δ^9-THC in the radial-arm maze task strongly indicates the involvement of CB1 cannabinoid receptors.

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References

Abel EL (1971) Retrieval of information after use of marihuana. Nature 231: 58

Carlini EA, Hamaoui A, Bieniek D, Korte F (1970) Effects of (-)Δ^9-*trans*-tetrahydrocannabinol and a synthetic derivative on maze performance of rats. Pharmacology 4: 359–368

Collins DR, Pertwee RG, Davies SN (1995) Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-meidated blockade of long-term potentiation in the rat hippocampal slice. Br J Pharmacol 115: 869–870

Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992a) Aminoalkylindole analogs: Cannabimimetic activity of a class of compounds structurally distinct from Δ^9-tetrahydrocannabinol. J Pharmacol Exp Ther 263: 1118–1126

Compton DR, Johnson MR, Melvin LS, Martin BR (1992) Pharmacological profile of a series of bicyclic cannabinoid analogs – classification as cannabimimetic agents. J Pharmacol Exp Ther 260: 201–209

Compton DR, Rice KC, Costa BRd, Razdan RK, Melvin LS, Johnson MR, Martin BR (1993) Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther 265: 218–226

Compton DR, Aceto MD, Lowe J, Martin BR (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exper Ther 277: 586–594

Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA, Axelrod J (1993) Anandamide, and endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. Pharmacol Biochem Behav 46: 967–972

Dennes RP, Barnes JC (1993) Attenuation of scopolamine-induced spatial memory deficits in the rat by cholinomimetic and non-cholinomimetic drugs using a novel task in the 12-arm radial maze. Psychopharmacology 111: 435–441

Deutsch DG, Chin SA (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 46: 791–796

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946–1949

Dewey WL (1986) Cannabinoid pharmacology. Pharmacol Rev 38: 151–78

Evans EB, Wenger GR (1992) Effects of drugs of abuse on acquistion of behavioral chains in squirrel monkeys. Psychopharmacology 107: 55–60

Ferraro DP, Grilly DM (1973) Lack of tolerance to Δ^9-tetrahydrocannabinol in chimpanzees. Science 179: 490–492

Ferri S, Costa G, Murari G, Panico AM, Rapisarda E, Speroni E, Arrigo RR (1981) Investigations on behavioral effects of an extract of Cannabis sativa L. in the rat. Psychopharmacology 75: 144–147

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantative in vitro autoradiographic study. J Neurosci 11: 563–583

Heyser CJ, Hampson RE, Deadwyler SA (1993) Effects of delta^9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. J Pharmacol Exp Ther 264: 294–307

Levin ED, Rose JE (1991) Interactive effects of D_1 and D_2 agonists with scopolamine on radial-arm maze peformance. Pharmacol Biochem Behav 38: 243–246

Lichtman AH, Dimen KR, Martin BR (1995) Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology 119: 282–290

Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR (1988) Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J Pharmacol Exp Ther 247: 1046–1051

Martin BR (1986) Cellular effects of cannabinoids. Pharmacol Rev 38: 45–74

Matsuoka N, Maeda N, Ohkubo Y, Yamaguchi I (1991) Differential effects of physostigmine and pilocarpine on the spatial memory deficits produced by two septo-hippocampal deafferentations in rats. Brain Res 559: 233–240

McConnell WR, Dewey WL, Harris LS, Borzelleca JF (1978) A study of the effect of delta^9-tetrahydrocannabinol (delta^9-THC) on mammalian salivary flow. J Pharmacol Exp Ther 206: 567–573

McGurk SR, Levin ED, Butcher LL (1991) Impairment of radial-arm maze performance in rats following lesions involving the cholinergic medial pathway: reversal by arecoline and differential effecs of muscarinic and nicotinic antagonists. Neuroscience 44: 137–147

McGurk SR, Levin ED, Butcher LL (1992) Dopaminergic drugs reverse the impairment of radial-arm maze performance caused by lesions involving the cholinergic medial pathway. Neuroscience 50: 129–135

Murray CL, Fibiger BC (1985) Learning and memory deficits after lesions of the nucleus basalis magnocellularis: reversal by physostigmine. Neuroscience 14: 1025–1032

Nakamura EM, da Silva EA, Concilio GV, Wilkinson DA, Masur J (1991) Reversible effects of acute and long-term adminstration of Δ^9-tetrahydrocannabinol (THC) on memory in the rat. Drug Alcohol Depend 28: 167–175

Olton DS (1987) The radial arm maze as a tool in behavioral pharmacology. Physiol Behav 40: 793–797

Paton WDM, Aboo Zar M (1968) The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J Physiol 194: 13–33

Pertwee RG, Ross TM (1991) Drugs which stimulate or facilitate central cholinergic transmission interact synergistically with Δ^9-tetrahydrocannabinol to produce marked catalepsy in mice. Neuropharmacology 30: 67–71

Prescott WR, Gold LH, Martin BR (1992) Evidence for separate neuronal mechanisms for the discriminative stimulus and catalepsy induced by Δ^9-THC in the rat. Psychopharmacology 107: 117–124

Revuelta AV, Cheney DL, Costa E, Lander N, Mechoulam R (1980) Reduction of hippocampal acetylcholine turnover in rats treated with (–)-delta 8-tetrahydrocannabinol and its 1′,2′-dimethyl-heptyl homolog. Brain Res 195: 445–452

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié P, Brelière JC, Le Fur G (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350: 240–244

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Alonso R, Congy C, Soubrié P, Brelière JC, Le Fur G (1995) Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci 56: 1941–1947

Tallarida RJ, Murray RB (1987) Manual of pharmacologic calculations with computer programs, 2nd edn. Springer, New York

Tilson HA, McLamb RL, Shaw S, Rogers BC, Pediatitakis P, Cook L (1988) Radial-arm maze deficits produced by colchicine administered into the area of the nucleus basalis are ameliorated by cholinergic agents. Brain Res 438: 83–94

Tripathi HL, Vocci FJ, Brase DA, Dewey WL (1987) Effects of cannabinoids on levels of acetylcholine and choline and on turnover rate of acetylcholine in various regions of the mouse brain. Alcohol Drug Res 7: 525–532

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994) Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63: 637–652

Wiley JL, Lowe J, Balster RL, Martin BR (1995a) Antagonism of the discriminative stimulus effects of Δ^9-THC in rats and rhesus monkeys. J Pharmacol Exp Ther 275: 1–6

Wiley JL, Barrett RL, Lowe J, Balster RL, Martin BR (1995b) Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology 34: 669–676