ELSEVIER

Progress in Neuro-Psychopharmacology & Biological Psychiatry 26 (2002) 321–325

Progress In Neuro-Psychopharmacology & Biological Psychiatry

SR 141716A prevents Δ^9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice☆

George E. Da Silva, Reinaldo N. Takahashi*

Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, UFSC, R. Ferreira Lima, 82, Florianópolis, SC 88015-420, Brazil

Abstract

This study reports a series of spatial discrimination procedures in a Morris-type maze to investigate the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on different phases of learning and memory in mice. Adult male mice were given training trails to find the submerged platform at a fixed location in the water maze adapted for mice. In additional experiments, mice were trained with the repeated acquisition procedure to test the working memory. Results indicate that Δ^9-THC (8 mg/kg ip) 30 min pretest impaired specifically the acquisition of spatial learning and the performance of mice in the working memory task, while consolidation and retrieval of a previously learned task were not affected. There was no evidence of motoric difficulty, as the number of quadrant line crossings was not decreased and no visible sign of sensorimotor disturbance was observed during swimming. Pretreatment with SR 141716A (1 mg/kg ip), a CB1 cannabinoid receptor antagonist, significantly prevented the learning deficits in the water maze. These findings show that Δ^9-THC impairs spatial discrimination learning in a selective way in the water maze in mice and that these deficits may be mediated by cannabinoid receptors.

Keywords: Cannabinoids; Morris water maze; Reference memory; Working memory

1. Introduction

The naturally occurring cannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), and synthetic agonists have been well documented to impair memory in animals and in humans (Carlini et al., 1970; Heyser et al., 1993; Hampson and Deadwyler, 1999). For example, in rodents, the acquisition and retention in the radial arm maze are profoundly impaired by acute Δ^9-THC administration (Nakamura et al., 1991; Lichtman and Martin, 1996; Lichtman et al., 1995). Moreover, deficits in working memory tasks have been reported in rats and monkeys (Ferraro and Grilly, 1973; Jentsch et al., 1997). The high concentration of cannabinoid CB1 receptors, the molecular target for marijuana derivatives, in hippocampus and medial prefrontal cortex (Herkenham et al., 1991; Gessa et al., 1998)—brain areas involved in the control of cognitive processes—suggest that a cannabinoid system may play a modulatory role in learning and memory. Indeed, more recent studies have shown that cannabinoids impair hippocampally mediated information processing critical to performance of different behavioral tasks (Hampson and Deadwyler, 1998, 1999).

Early studies of the spatial memory abilities of rodents employed a variety of mazes types, but a more recently developed memory task is the Morris water maze (Morris, 1984), which has proven very useful and has shown to offer several advantages over other methods of studying the neurochemical basis of learning and memory in rodents (Brandeis et al., 1989). Thus, the standard water escape task, in which an animal is required to localize a submerged platform, measures predominantly spatial reference memory, while repeated acquisition procedures are designed to assess an additional memory component—the working memory (Barnes, 1988).

Although cannabinoid CB1 receptors knockout (KO) mice have been recently generated (Ledent et al., 1999), to our knowledge, little information is available concerning the effects of cannabinoids on this type of learning in the

☆ Portions of these data were presented at the XXIInd CINP Congress, Brussels, July 9–13, 2000.

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; SR 141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl.

* Corresponding author. Tel.: +55-48-331-9764; fax: +55-48-222-4164.
E-mail address: takahashi@farmaco.ufsc.br (R.N. Takahashi).

0278-5846/02/$ – see front matter
PII: S0278-5846(01)00275-5

mouse. In view of these considerations and given certain practical advantages of using the mouse, a series of spatial discrimination procedures was used in a Morris-type water maze to investigate the effects of Δ^9-THC on learning/memory processes in mice. In addition, the effects of SR 141716A, the CB1 cannabinoid receptor antagonist, pretreatment on Δ^9-THC-induced memory impairments were evaluated in the water maze.

2. Materials and methods

2.1. Animals

All procedures were in strict accordance with the guidelines of the UFSC University Animal Care Committee. Male albino mice weighing approximately 25–30 g were used. They were housed in groups with ad libitum access to food and water. A 12-h light/dark cycle was maintained (0700–1900 h) with all testings carried out between 0900 and 1400 h.

2.2. Drugs

Δ^9-THC was provided by the National Institute on Drug Abuse (USA) and SR 141716A was a gift from SANOFI Recherche (France). The appropriate concentration of Δ^9-THC was prepared by evaporating the alcohol and emulsifying the residue with Tween-80 (Takahashi and Singer, 1979). One drop of Tween-80 for 10 ml was added for the preparation of the SR 141716A suspension. Control solution was prepared with the corresponding vehicle. All solutions were administered by intraperitoneal route in a volume of 1 ml/kg.

2.3. Apparatus

The water maze consisted of a circular white plastic pool (70 cm diameter, 60 cm in depth) filled with water. The maze was located in a room containing many extra-maze cues. Four starting positions (north—N, south—S, east—E, west—W) were equally spaced around the perimeter of the task, dividing the pool into four quadrants. A 7×7 cm transparent platform was submerged at a depth of 0.5 cm below the surface of the water. To obtain an arbitrary measure of the distance swum, quadrant lines were defined.

2.4. Experimental procedure

In the experiment aimed to examine the acquisition of spatial reference memory, mice received a four-trail training session per day to find a submerged platform located in the same quadrant of the water maze. Δ^9-THC (4, 6 or 8 mg/kg) or control solution was injected 30 min before the daily sessions during 7 days. Additional groups of animals received identical training, but were injected with Δ^9-THC 30 min after the daily sessions (consolidation). To examine the retrieval of previously learned spatial information, groups of trained mice were injected with the treatment just once, 30 min before Session 7.

In the working memory procedure, each mouse was required to find the submerged platform located in a new position each day. The location of the hidden platform was changed daily in a pseudorandomic way, such as different mice were tested in all quadrants on given day and all mice were trained in each quadrant at least twice during the experiment. One day after reaching the acquisition criterion, escape latency time less than 15 s, mice were injected with Δ^9-THC or control solution and the working memory test performed 30 min later. In the pretreatment studies, the animals were injected with SR 141716A (1 mg/kg) 15 min before the treatment. The latency to mount the escape platform and the number of quadrant line crossings were recorded manually.

2.5. Data analysis

Data were analyzed using two-way analysis of variance (ANOVA) for repeated measures. Subsequent comparisons were done using the Newman–Keuls' posthoc tests. Student's t test was used to analyze the results of working memory experiment. Values of $P < .05$ were considered significant.

3. Results

All mice were capable of swimming around the pool until the platform was located and then climbing onto it. The results of the effects of Δ^9-THC, 4, 6 or 8 mg/kg, on latencies to find the submerged platform in the water maze are presented in Fig. 1. As can be seen, all animals showed a progressive decline in escape latency with training. A two-way ANOVA of escape latencies for the

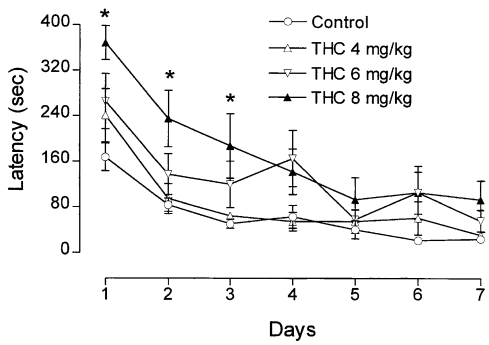

Fig. 1. Effects of different doses of Δ^9-THC on latencies (s) to find the submerged platform in the Morris water maze. Animals were injected intraperitoneally with Δ^9-THC or control solution daily, 30 min before each session. Mean ± S.E.M. is shown for each group of 8–12 animals. * Significantly different from control group; $P < .05$, Newman–Keuls' test.

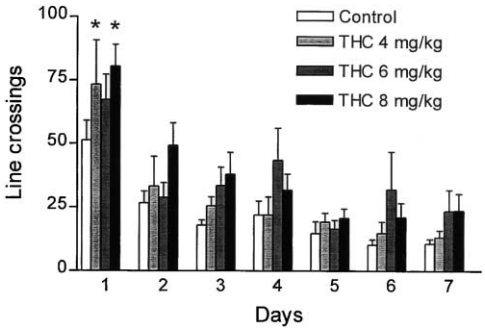

Fig. 2. Effects of different doses of Δ^9-THC on means number of quadrant line crossings in the water maze. Mean ± S.E.M. is shown for each group of 8–12 animals. * Significantly different from control group; $P < .05$, Newman–Keuls' test.

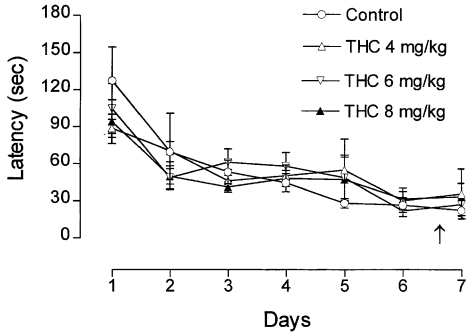

Fig. 4. Effects of Δ^9-THC on the retrieval of acquired spatial information in the water maze. Animals were injected intraperitoneally with Δ^9-THC (4, 6 or 8 mg/kg) or control solution, 30 min before seventh session (black arrow). Mean ± S.E.M. is shown for each group of seven animals.

training days revealed significant Group differences [$F(3,36) = 4.90$, $P < .01$] and Day effect [$F(6,216) = 34.08$, $P < .00001$]. Subsequent comparisons using Newman–Keuls' tests showed that Δ^9-THC (8 mg/kg) group performed significantly worse than the other groups mainly over the early days ($P < .05$). A two-way ANOVA computed on number of line crossings revealed only a significant effect of Group factor [$F(3,36) = 2.77$, $P < .05$]. Further comparisons using posthoc tests indicated that only on Day 1 did Δ^9-THC treatment significantly increase the number of crossings (Fig. 2). Regardless of whether this parameter is operationally considered as the distance swum and/or the swimming ability, it seems clear that the treatment did not cause motor deficit of mice tested in the pool.

The effect of SR 141716A pretreatment on the Δ^9-THC-induced impairment on spatial reference memory is shown in Fig. 3. A two-way ANOVA of escape latencies for the training days revealed a significant effect of Groups [$F(3,35) = 6.47$, $P < .001$], Days [$F(6,210) = 33.41$, $P < .00001$] and a Groups × Days interaction [$F(18,210) = 2.141$, $P < .005$]. Post-hoc comparisons showed that SR 141716A completely blocked the disruptive effects of Δ^9-THC in the water maze, as indicated by the return to baseline levels (Fig. 3). There were no significant differences in the number of quadrant line crossings among these groups (data not show).

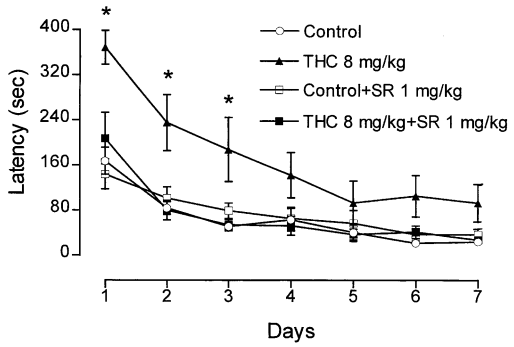

Fig. 3. Effects of Δ^9-THC (8 mg/kg), SR 141716A (1 mg/kg) and the combination Δ^9-THC + SR 141716A on latencies (s) to find the submerged platform in the water maze. Animals were injected intraperitoneally with the treatments daily, 30 min before each session. Mean ± S.E.M. is shown for each group of 8–12 animals. * Significantly different from control group; $P < .05$, Newman–Keuls' test.

The effect of posttraining administration of Δ^9-THC (4, 6 or 8 mg/kg) on retrieval of spatial discrimination task is shown in Fig. 4. A two-way ANOVA revealed significant effects only for Days factor [$F(6,144) = 20.523$, $P < .00001$, for escape latencies; $F(6,144) = 12.366$, $P < .00001$, for the number of line crossings]. All groups of mice exhibited similar learning curves during the training sessions. At the end of Day 6 of training, the mice assigned to control or

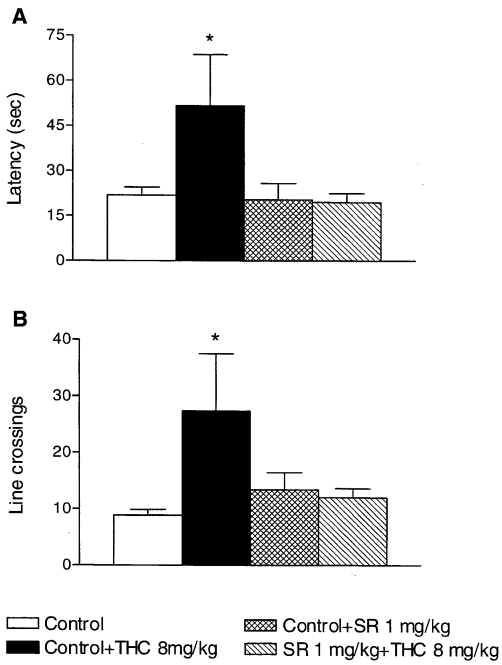

Fig. 5. Effects of Δ^9-THC (8 mg/kg) on working memory evaluated in the water maze. (A) Mean latency to find the submerged platform. (B) Mean number of quadrant line crossings. Animals were tested with different treatments only after reaching the acquisition criterion. The intraperitoneal injections were made 30 min before testing. Mean ± S.E.M. is shown for each group of seven to eight animals. * Significantly different from control group; $P < .05$, Student's t test.

Δ^9-THC showed the same pattern of response (Fig. 4). These findings indicate that posttraining injection of Δ^9-THC on Day 7 did not affect the retrieval of previously acquired spatial information in the maze. There was no significant difference in the consolidation of spatial discrimination task between control and mice treated with Δ^9-THC during 7 days (data not shown).

The results of mice tested in the working memory test are depicted in Fig. 5. After 10–14 days of training, all mice began to reach the criterion established in this procedure. The test was conducted in the following day with administration of treatment 30 min before the experiments. As can be seen, Δ^9-THC-injected mice required significantly more time to find the platform and crossed more lines compared to control group (Student's t test, $P<.05$). Also illustrated in Fig. 5, there was a trend for SR 141716A to prevent the impaired cognitive performance induced by Δ^9-THC, but this tendency did not reach statistical significance ($P<.06$). It is important to note that at the dose used presently, SR 141716A did not alter these measures on its own.

4. Discussion

The series of experiments reported here indicates that systemic administration of Δ^9-THC produces both a deficient acquisition of spatial reference memory and of the working memory evaluated in a Morris-type water maze in mice. The same dose of Δ^9-THC was without effect on performance related to consolidation and retrieval. The deficit of place learning induced by Δ^9-THC was prevented by SR 141716A, a CB1 cannabinoid receptors antagonist.

Δ^9-THC (8 mg/kg) significantly increased the latency to reach the submerged platform, the most frequently used measure for learning in the water maze, in two tasks, one involving the standard water escape and the other involving repeated acquisition procedure designed to assess the so-called working memory. These findings are in accordance with published reports of spatial learning deficits induced predominantly in rats and using other types of maze (Nakamura et al., 1991; Lichtman et al., 1995). The prolonged escape latency time caused by Δ^9-THC could be attributed to its known locomotor suppressive effects. However, some observations argue against this alternative interpretation. The number of quadrant line crossings was increased or unaffected by Δ^9-THC; further, acquisition, consolidation and retrieval should be affected in the same way and the absence of motor incoordination induced by Δ^9-THC at the same dose range on the rotarod performance (unpublished data). Thus, the present results provide strong evidence that Δ^9-THC induces a selective impairment of spatial memory independent of its locomotor effects.

The acquisition impairment may be the result of activation of cannabinoid receptors by Δ^9-THC, since the CB1 receptor antagonist SR 141716A attenuated these effects. These findings further support the notion that memory impairment induced by Δ^9-THC is mediated by CB1 receptors (Lichtman and Martin, 1996; Mallet and Beninger, 1998). As mentioned in the Introduction, the cognitive disrupting effects of Δ^9-THC can be attributed to the high density of cannabinoid receptors localized not only in the hippocampus (Herkenham et al., 1991), but also in the prefrontal cortex area (Jentsch et al., 1997).

While cannabinoid system seems to be involved in the acquisition phase, the same treatment did not affect the retrieval of consolidated spatial information. This view is supported by the study of Nakamura et al. (1991), where Δ^9-THC unaffected the retrieval of a previously learned spatial task in a radial arm maze in rats. It is possible that learning can be more easily disrupted than retrieval of a learned response or simply an indication that acquisition and retrieval are qualitatively different processes with regard to neurochemical events.

Although mice are used less frequently in Morris water maze studies, the present results also confirm that mice can readily learn the maze task and the potential advantages, such as more extensive use of inbred or transgenic mice in behavioral pharmacology, for example, to assess cognitive drugs cannot be ignored (Klapdor and Staay, 1996).

5. Conclusion

The results reported here demonstrate for the first time that Δ^9-THC induces a selective impairment of spatial reference memory and working memory in mice tested in a Morris-type water maze. In addition, these deficits are prevented by cannabinoid receptors antagonist.

Acknowledgments

This work was partially supported by CNPq Brasil.

References

Barnes, C.A., 1988. Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. Trends Neurosci. 11, 163–169.

Brandeis, R., Brandys, Y., Yehuda, S., 1989. The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci. 48, 29–69.

Carlini, E.A., 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.

Ferraro, D.P., Grilly, D.M., 1973. Lack tolerance to Δ^9-tetrahydrocannabinol in chimpanzees. Science 179, 490–492.

Gessa, G.L., Casu, M.A., Carta, G., Mascia, M.S., 1998. Cannabinoids decrease acetylcholine release in the medial pre-frontal cortex and hippocampus, reversal by SR 141716A. Eur. J. Pharmacol. 355, 119–124.

Hampson, R.E., Deadwyler, S.A., 1998. Role of cannabinoid receptors in memory storage. Neurobiol. Dis. 5, 474–482.

Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoids, hippocampal function and memory. Life Sci. 65 (6–7), 715–723.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., De Costa, B.R., Rice K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Heyser, C.J., Hampson, R.E., Deadwyler, S.A., 1993. Effects of Δ^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.

Jentsch, J.D., Andrusiak, E., Tran, A., Bowers, M.B., Roth, R.H., 1997. Δ^9-Tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rat: blockade of dopaminergic effects with HA966. Neuropsychopharmacology 16, 426–432.

Klapdor, K., Staay, J.V.D., 1996. The Morris water-escape task in mice: strain differences and effects of intra-maze contrast and brightness. Physiol. Behav. 60, 1247–1254.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.-F., Baslet, F., Bohme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knock-out mice. Science 283, 401–404.

Lichtman, A.H., Martin, B.R., 1996. Δ^9-Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology 126, 125–131.

Lichtman, A.H., Dimen, K.R., Martin, B.R., 1995. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology 119, 282–290.

Mallet, P.E., Beninger, R.J., 1998. The cannabinoid CB1 receptor antagonist SR 141716A attenuates the memory impairment produced by Δ^9-tetrahydrocannabinol or anandamide. Psychopharmacology 140, 11–19.

Morris, R.G.M., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60.

Nakamura, E.M., Da Silva, E.A., Concílio, G.V., Wilkinson, D.A., Masur, J., 1991. Reversible effects of acute and long-term administration of Δ-9-tetrahydrocannabinol (THC) on memory in the rat. Drug Alcohol Depend. 28, 167–175.

Takahashi, R.N., Singer, G., 1979. Self-administration of Δ^9-tetrahydrocannabinol by rats. Pharmacol. Biochem. Behav. 11, 737–740.