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Effects of chronic Δ^9-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze

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Abstract

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the psychoactive ingredient of *cannabis sativa*, reduces both extracellular hippocampal acetylcholine concentration and correct alternation tasks in the T-maze.

The principal aim of this study was to determine whether a chronic Δ^9-THC treatment would induce tolerance both to the reduction of extracellular hippocampal acetylcholine concentration and memory deficit produced by the drug.

Our results show that a chronic Δ^9-THC treatment (5 mg/kg, i.p., twice daily for two weeks) did not produce tolerance to the inhibitory effects induced by the drug. Moreover, no strict temporal correlation between the two Δ^9-THC effects was observed: the inhibition in extracellular acetylcholine concentration appeared only 80 min after treatment, while the reduction of correct alternation tasks in the T-maze began after 20 min. The cognitive and cholinergic effects induced by a chronic Δ^9-THC treatment were completely blocked by the CB_1 cannabinoid receptor antagonist SR 141716A, indicating an involvement of CB_1 cannabinoid receptors in the persistent negative effects induced by the drug.

These findings confirm the proposition that CB_1 cannabinoid receptors mediate the negative effects induced by Δ^9-THC both on hippocampal extracellular acetylcholine concentration and correct alternation tasks in the T-maze, and they indicate that these effects may be differentiated. However, the major outcome of this work is the demonstration that no tolerance to the two inhibitory effects develops after a chronic Δ^9-THC treatment. © 2001 Published by Elsevier Science Ltd.

Keywords: Cannabinoids; Microdialysis; T-maze

1. Introduction

Marijuana and its active ingredient Δ^9-tetrahydrocannabinol (Δ^9-THC) impair learning and memory processes in rodents (Heyser et al., 1993; Terranova et al., 1996; Brodkin and Moerschbaecher, 1997; Jentsch et al., 1997; Nava et al., 2000), non-human primates (Evans, 1992) and humans (Miller and Branconnier, 1983; Chait and Perry, 1994; Heishman et al., 1997). Accordingly, the synthetic cannabinoids {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinylmethyl] pyrol [1,2,3-de]1,4-benzoxazin-6-yl) (l-naphthalenyl) methanone monomethanesulfonate} (WIN 55,212-2) and {[1a,2-(r)-5-(1,1-dimethylpheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-phenol} (CP 55,940), and the endogenous CB_1 cannabinoid receptor agonist anadamide have been also shown to impair memory processes in rodents (Lichtman et al., 1995; Lichtman and Martin, 1996; Mallet and Beninger, 1998). The cognitive impairment induced by cannabinoids is antagonized by the CB_1 cannabinoid receptor antagonist *N*-(piperidine-l-yl)-5-(4-chlorophenyl)-l-(2,4-dichlorophenyl)-4-methyl-lH-pyrazole-3-carboxamide hydrochloride (SR 141617A) (Lichtman and Martin, 1996). Moreover, recent data have shown an enhancement of memory processes in CB_1 cannabinoid receptors knock-out mice (Reibaud et al., 1999). Taken together, this evidence indicates a possible involvement of CB_1 cannabinoids receptors in cognitive functions (Lichtman and Martin, 1996; Reibaud et al., 1999).

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Acetylcholine is a crucial neurotransmitter contributing to attentional (Perry et al., 1998) and memory function (Aigner, 1995). Moreover, different studies have shown that CB_1 cannabinoid receptors are abundant in the hippocampus (Pertwee, 1997; Herkenham, 1995), a primary area involved in learning and memory processes (Vizi and Kiss, 1998). Some evidence has shown that cannabinoids impair working memory after intrahippocampal administration (Lichtman et al., 1995), inhibit long-term potentation in hippocampal slices (Collins et al., 1995) and reduce hippocampal acetylcholine concentration both in vivo (Carta et al., 1998; Gessa et al., 1997; Gessa et al., 1998a; Nava et al., 2000) and in vitro (Gifford and Ashby, 1996; Gifford et al., 2000). These data suggest that hippocampal cholinergic activity may mediate cannabinoid effects on memory, although Lichtman and Martin (1996) have shown that the physostigmine, an inhibitor of acetylcholisterase, does not block the inhibition of working memory induced by Δ^9-THC. In accordance with previous data, a recent study from our group has demonstrated that, after an acute Δ^9-THC treatment, the inhibition of extracellular hippocampal acetylcholine concentration is not temporally correlated to the reduction of correct alternation tasks in the T-maze (Nava et al., 2000). Alternatively, it has been suggested that the negative effects induced by cannabinoids on working memory are due to dopaminergic hyperactivity in the brain (Jentsch et al., 1997). This hypothesis is supported by three findings. The first being that cannabinoids activate meso-limbic and meso-cortical dopamine neurons (Diana et al., 1998; Gessa et al., 1998b) and increase release (Chen et al., 1990) and turnover of dopamine in the prefrontal cortex (Jentsch et al., 1997). The second that Δ^9-THC-induced memory impairment and increase in dopamine turnover are antagonized by 3-amino-l-hydroxy-2-pyrrolidone (HA 966) (Jentsch et al., 1997), a compound that inhibits the activity of dopaminergic neurons (Shepard et al., 1995). The third that S(−)-sulpiride, a D_2 dopamine receptor antagonist, blocks both the inhibition of hippocampal acetylcholine concentration and the reduction of correct alternation tasks in the T-maze induced by Δ^9-THC (Nava et al., 2000).

The studies on chronic effects of marijuana, including tolerance phenomena, are relevant to evaluate the harm associated with the use of cannabinoids in medicine and to elucidate the mechanisms underlying the cognitive deficits induced by cannabis abuse. It is not yet clear whether a long-term treatment with Δ^9-THC produces tolerance to the ability of the drug to affect the hippocampal cholinergic transmission and to impair memory performances. Several studies have shown that acutely administered marijuana impairs cognition in humans (Hall and Solowij, 1998; Hollister, 1986).

In the light of the above evidence, the principal aim of this study was to determine whether the detrimental effects of Δ^9-THC both on hippocampal extracellular acetylcholine concentration and correct alternation tasks in the T-maze are still present after a chronic drug treatment.

2. Material and methods

2.1. Animals

Male Sprague Dawley rats (200–250 g; Charles River, Como, Italy) were housed individually in a Plexiglass chamber (height 25, length 40 and width 15 cm) at 22+1°C and 55% of humidity. Food and water were freely available and animals were maintained under an artificial 12 h/12 h light/dark schedule with lights on from 08:00 to 20:00. Experiments were carried out between 09:00 and 17:00. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Experimental procedure

The effects of Δ^9-THC on extracelluar acetylcholine concentration were studied in freely moving rats by microdialysis, while the effects on working memory were studied in a separate group of unoperated animals trained to correctly perform a delayed alternation task in the T-maze.

2.3. Surgery and microdialysis

Implantation of the microdialysis probes was performed under general anaesthesia (equithensin 4 ml/kg, i.p.). Briefly, rats were implanted with a trasversal dialysis probe (AN 69-HF, tube outer diameter 320 μm; Hospal-Dasco, Bologna, Italy), passing bilaterally through the hippocampi (A=−3.3 and V=−3.6; A and V being referred to bregma and skull, respectively), according to the atlas of Paxinos and Watson (1986). The dialysis probe had an active dialysis length of 0.8 cm.

Upon completion of the experiments each rat was sacrificed and the location of the probe was verified histologically. Only data from rats with a proper location of the probe were used.

Microdialysis perfusion was performed 24 h after implantation. The probe was perfused at a constant rate of 2 μl/min with a Ringer solution containing (mM) KCl 3.0, NaCl 125, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $NaHCO_3$ 23, in potassium phosphate 1.5 and pH 7.3. Neostigmine bromide was added at a final concentration of 0.1 mM in order to recover detectable concentrations of dialysate acetylcholine. Samples were collected every 20 min, corresponding to a volume of 40 μl, and were immediately injected in a high-performance liquid chromatography (HPLC) system with electrochemical detection accord-

ing to the technique described by Damsa and Westerink (1991). The detection limit for acetylcholine was 0.05 fmol per 1 μl of sample. Samples were collected every 20 min (40 μl) and the average concentration of acetylcholine in the last three pre-drug samples, obtained after 1 h of perfusion, was taken as 100%. All subsequent post-treatment values were expressed as percent (mean+S.E.M.) of basal values.

2.4. Delayed alternation testing in the T-maze

Working memory was evaluated in a standard T-maze made of black plexiglass, and consisting of a central stem (height 10, length 40 and width 20 cm) with a start box (the first 20 cm of the stem) and two arms (height 20, length 60 and width 20 cm) with a wire mesh floor. The start compartment and each goal arm were separated from the central stem by a guillotine door. Complete entrance into the arm was required in order to enable animals to reach the food cup. The T-maze was located in a dimly illuminated room and a weak light (25 W) was positioned 30 cm over the right-hand food cup in order to allow rats to distinguish between the right and left arms by brightness.

Training and testing were performed according to the method described previously by Nava et al. (2000).

Seven days prior to starting the training session, animals were housed individually in a Plexiglass chamber (height 25, length 40 and width 15 cm) at 22+1°C and 55% of humidity. To increase their motivation for food, animals were maintained at 85% of their pre-experimental body weight by feeding them a limited amount of chow (see later). Water was always available in the home cage.

A daily training session consisted of eleven consecutive trials, in which the rat had to alternate between the right and left arm of the maze to obtain a reward consisting of a shelled sunflower seed. During the first trial of each session, access to one of the two arms was blocked forcing the rat to enter the other arm; the direction of the forced trial was alternated daily. On each of the consecutive ten trials the food was placed in the arm opposite the previous correct trial, and both arms were unblocked (free-choice trials). A correct trial ended with the rat eating the food. An incorrect trial ended with the rat reaching the empty food cup. If the rat did not enter into an arm within 2 min the trial was not counted and the rat was given another attempt. After each trial, the rat was removed from the goal arm and kept in the start compartment for a delay period after which the door of central stem was opened.

The data collected from each session were analysed in terms of percentage of correct alternation choices in the T-maze.

A delay length of 8 s, in which rats' memory performance was stabilized at 90% of correct alternation tasks in the T-maze, was used. The criterion defining a correct training session was a successful daily trial for three consecutive days. All drugs were injected to animals attaining the above criterion.

For each drug treatment the order of dose administration was counter-balanced among the subjects in a latin-square design. In particular, in order to reduce inter-group variables, for each group of rats, we used the same animals tested at each time after a washout period of 1 week during which they received no drug and re-established criterion performance (see earlier).

2.5. Drugs

Δ^9-THC (RBI, Italy) solutions were prepared from vials containing 10 mg in 1 ml of absolute ethanol. Δ^9-THC was evaporated under nitrogen and the residue dissolved in two drops of Tween 80 and then diluted in saline. The specific CB_1 cannabinoid receptor antagonist SR 141617A (Sanofi Recherche, Montpellier, France) was dissolved in two drops of Tween 80 and then diluted in saline. Δ^9-THC and SR 141617A were administered intraperitoneally (i.p.) in a volume of 1 ml/kg. Control rats were treated with the vehicle (1 ml/kg, i.p.) used to dissolve the active ingredient.

2.6. Statistical analysis

Within-group comparison was performed by means of analysis of variance (ANOVA) for repeated measures, while between-group comparisons and time×group comparisons were performed by a two-way analysis of variance (ANOVA). Post-hoc comparison was carried out using a Tukey test. Significance was established at $P<0.01$.

3. Results

3.1. Acute treatment

3.1.1. Extracellular hippocampal acetylcholine concentration

In agreement with previous results (Carta et al., 1998; Gessa et al., 1997; Gessa et al., 1998a; Nava et al., 2000), Δ^9-THC at the dose of 2.5 and 5 mg/kg, i.p., inhibited the extracellular hippocampal acetylcholine concentration (Fig. 1). A significant inhibition appeared at 80 min after treatment and was maximal at 120 min, being no longer present at 12 h after injection (Fig. 1).

The Δ^9-THC-induced inhibition of extracellular acetylcholine concentration was reverted by the CB_1 cannabinoid receptor antagonist SR 141716A (0.5 mg/kg, i.p.), given 20 min after Δ^9-THC treatment (Fig. 2). SR

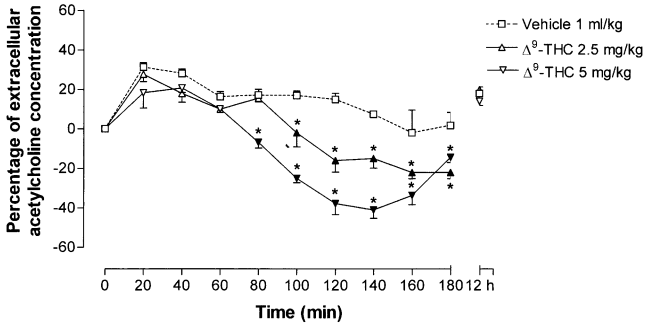

Fig. 1. Δ^9-THC reduced hippocampal acetylcholine concentration at the dose of 2.5 and 5 mg/kg, i.p. (main effect of treatment $F_{2.12}$=10.20, P<0.01; main effects of repeated measures $F_{9.18}$=23.6, P<0.0001; time×group interaction $F_{9.18}$=2.9, P<0.001). Filled signs P<0.01 compared with basal values (Tukey test) and asterisks signs P<0.01 compared with vehicle treated rats (Tukey test). Data are expressed as percentage (mean+S.E.M.; n=5) of the respective baseline values. Prior the drug administration basal values of extracellular acetylcholine concentrations were: 1.38+0.13, 1.23+0.34 and 1.35+0.28 fmol/μl in animals treated with vehicle and Δ^9-THC at the doses of 2.5 and 5 mg/kg, respectively.

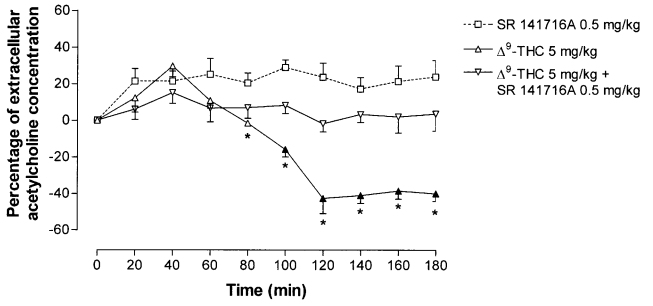

Fig. 2. Antagonism by SR 141716A of Δ^9-THC-induced reduction of extracellular hippocampal acetylcholine concentration (main effect of treatment $F_{2.12}$=10.20, P<0.005; main effect of repeated measures $F_{9.18}$=23.6, P<0.001; time×group interaction $F_{9.18}$=2.9, P<0.001). Filled signs P<0.01 compared with basal values (Tukey test) and asterisks signs P<0.01 compared with vehicle treated rats (Tukey test). Data are expressed as percentage (mean+S.E.M.; n=5) of the respective baseline values. Prior the drug administration basal values of extracellular acetylcholine concentrations were 1.42+0.15, 1.33+0.45 and 1.56+0.23 fmol/μl in rats treated with Δ^9-THC, SR 141716A or Δ^9-THC+SR 141716A, respectively.

141716A (0.5 mg/kg, i.p.), when given alone, did not modify extracellular acetylcholine concentration (Fig. 2).

3.1.2. Delayed alternation tasks in the T-maze

Δ^9-THC at doses of 2.5 and 5 mg/kg, i.p., reduced the percentage of correct alternation tasks in the T-maze 20 min after treatment (Fig. 3). The maximal reduction was observed at 60 min and no effect was present at 12 h (Fig. 3).

As shown in Fig. 4 Δ^9-THC-induced memory impairment was antagonized by the CB_1 cannabinoid receptor antagonist SR 141716A (0.5 mg/kg, i.p.), given 20 min before Δ^9-THC treatment. When given alone, SR

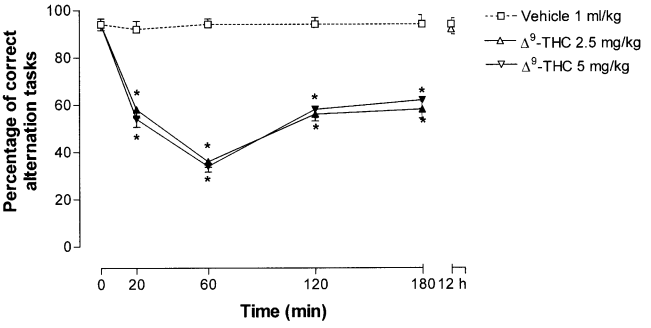

Fig. 3. Δ^9-THC reduced the correct alternation tasks in the T-maze at the doses of 2.5 and 5 mg/kg, i.p. (main effect of treatment $F_{2.12}$=181.3, P<0.0001; main effect of repeated measures $F_{4.8}$=66.2, P<0.0001; time×group interaction $F_{4.8}$=16.6, P<0.0001). Filled signs P<0.01 compared with basal values (Tukey test) and asterisks signs P<0.01 compared with vehicle treated rats (Tukey test). Data are expressed as percentage of correct alternation tasks in the T-maze (means+S.E.M.; n=5). Inter-trial delay was fixed at 8 s.

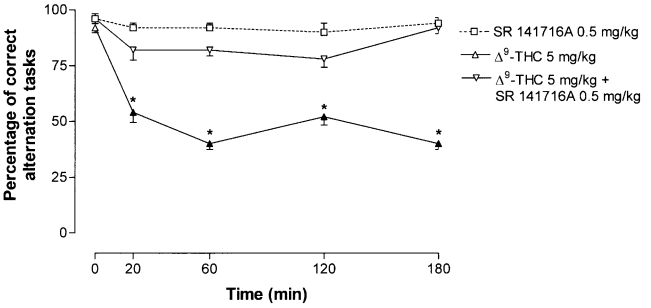

Fig. 4. Antagonism by SR 141716A of Δ^9-THC reduction of correct alternation tasks in the T-maze (main effect of treatment $F_{1.18}$=525.1, P<0.0001; main effect of repeated measures $F_{4.4}$=28.7, P<0.0001; time×group interaction $F_{4.4}$=13.6, P<0.0001). Filled signs P<0.01 compared with basal values (Tukey test) and asterisks signs P<0.01 compared with vehicle treated rats (Tukey test). Data are expressed as percentage of correct alternation tasks in the T-maze (means+S.E.M.; n=5). Inter-trial delay was fixed at 8 s.

141716A (0.5 mg/kg, i.p.) failed to modify rats' memory performance in the T-maze (Fig. 4).

Δ^9-THC did not significantly modify the amount of time required by animals to complete a T-maze session (Fig. 5).

3.2. Chronic treatment

3.2.1. Extracellular hippocampal acetylcholine concentrations

Two different groups of rats were treated with Δ^9-THC (5 mg/kg, i.p) or vehicle (1 ml/kg, i.p.), respectively, twice daily for 14 days. On the 14th day of treatment all rats were implanted with a microdialysis probe. On the 15th day from the first dose, animals were treated with a challenge dose of Δ^9-THC (2.5 mg/kg, i.p.). The extracellular hippocampal acetylcholine concentration was measured at various times after drug treatment (Fig. 6).

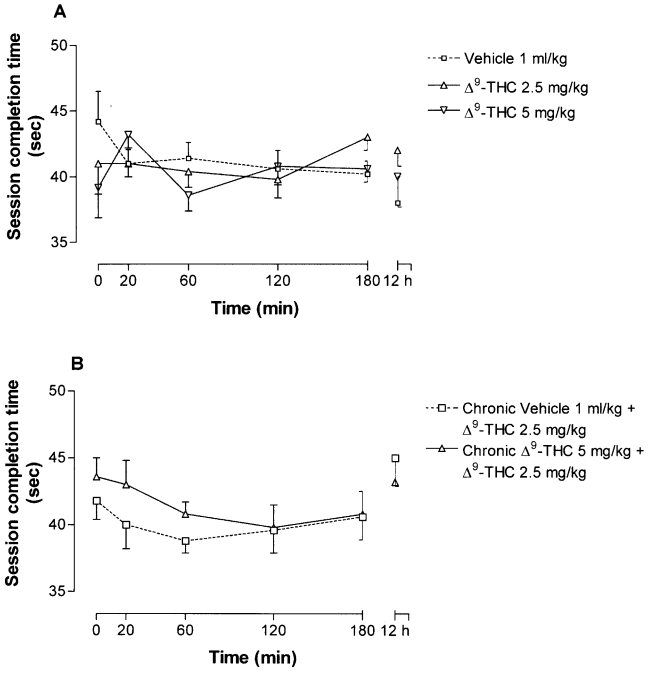

Fig. 5. Failure by an acute (A) (main effect of treatment $F_{2.12=}1.2$, $P>0.05$; main effects of repeated measures $F_{4.8=}0.9$, $P>0.05$; time×group interaction $F_{4.8=}1.4$, $P>0.05$) or chronic (B) (main effect of treatment $F_{1.8=}2.6$, $P>0.05$; main effects of repeated measures $F_{4.4=}1.3$, $P>0.05$; time×group interaction $F_{4.4=}3.7$, $P>0.05$) Δ^9-THC treatment to modify the amount of time required to the animals to complete a T-maze session. Filled signs $P<0.01$ compared with basal values (Tukey test). Data are expressed as time spent to complete a T maze session (means+S.E.M.; n=5), 60 min after Δ^9-THC treatment.

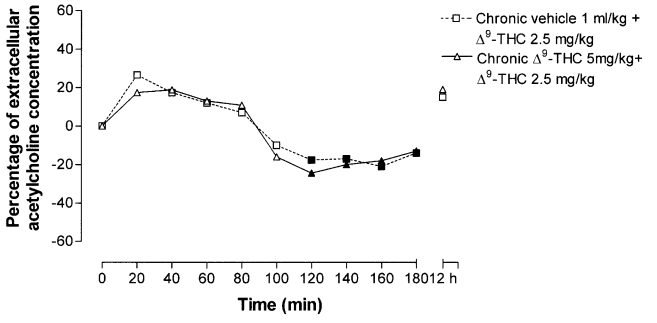

Fig. 6. Lack of tolerance to Δ^9-THC effect on extracellular hippocampal acetylcholine concentration (main effects of treatments $F_{1.8}$=2.4, $P>0.05$; main effects of repeated measures $F_{9.9}$=123.6, $P<0.001$; time×group interaction $F_{9.9=}2.2$, $P>0.05$). Filled signs $P<0.01$ compared with basal values (Tukey test). Data are expressed as percentage of the respective baseline values (means+S.E.M.; n=5). Prior the challenge dose of Δ^9-THC the basal values of extracellular acetylcholine concentrations were: 1.28+0.24 and 1.53+0.18 fmol/μl in rats treated chronically with vehicle or Δ^9-THC, respectively.

As shown in Fig. 7, the challenge dose of Δ^9-THC (2.5 mg/kg, i.p.) produced the same reduction in extracellular acetylcholine concentration both in animals treated chronically with Δ^9-THC as in those treated chronically with vehicle. The time-course of Δ^9-THC effects were identical in both groups of rats (Fig. 6).

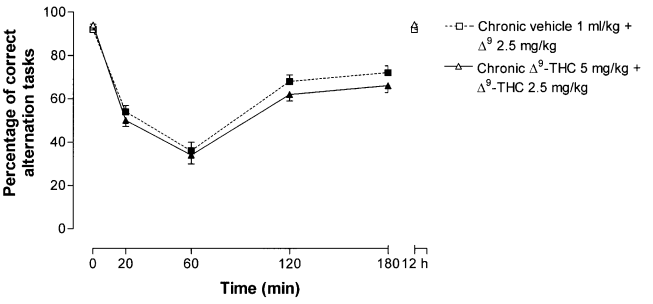

Fig. 7. Lack of tolerance to Δ^9-THC effect on reduction of correct alternation tasks in the T-maze (main effects of treatments $F_{1.8}$=3.2, $P>0.05$; main effects of repeated measures $F_{4.4}$=92.2, $P<0.001$; time×group interaction $F_{4.4=}0.6$, $P>0.05$). Filled signs $P<0.01$ compared with basal values (Tukey test). Data are expressed as percentage of correct alternation tasks in the T-maze (means+S.E.M.; n=5). Inter-trial delay was fixed at 8 s.

3.2.2. Delayed alternation tasks in the T-maze

Two different groups of rats trained to perform successfully three consecutive daily sessions in the T-maze were treated with Δ^9-THC (5 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.), respectively, twice daily for 14 days. Every day, 12 h from the evening dose in all groups of rats memory performance was not found to be impaired (data not shown). On the 15th day from the first dose, both groups of animals received a challenge dose of Δ^9-THC (2.5 mg/kg, i.p.). The choice accuracy in the T-maze alternation tasks was evaluated both before and at various times after drug treatment (Fig. 7).

As shown in Fig. 7, a challenge dose of Δ^9-THC (2.5 mg/kg, i.p.) produced the same reduction in correct alternation tasks in the T-maze both in rats chronically treated with Δ^9-THC and in those chronically treated with vehicle. The time course of Δ^9-THC effects were the same in both groups of animals (Fig. 7).

Rats chronically treated with Δ^9-THC or vehicle did not significantly modify their locomotor activity in the T-maze (Fig. 5).

4. Discussion

The present results confirm previous observations showing that Δ^9-THC, similar to other cannabinoids, inhibits extracellular hippocampal acetylcholine concentration both in vivo (Gessa et al., 1997; 1998a; Carta et al., 1998; Nava et al., 2000) and in vitro (Gifford and Ashby, 1996) and impairs memory (Lichtman et al., 1995; Lichtman and Martin, 1996; Mallet and Beninger, 1998; Jentsch et al., 1997; Nava et al., 2000). These effects were antagonized by the CB_1 cannabinoid receptors antagonist SR 141716A, indicating that they are mediated by the CB_1 cannabinoid receptors (Gessa et al., 1997; Carta et al., 1998; Lichtman and Martin, 1996; Nava et al., 2000).

The first finding of the present study is that the

reduction of both extracellular acetylcholine concentration and correct alternation tasks in the T-maze produced by Δ^9-THC challenge after a chronic drug administration is not time correlated, similar to observations made after acute treatment (Carta et al., 1998; Nava et al., 2000). Indeed, although the doses of Δ^9-THC producing cognitive impairment were the same as those which inhibited extracellular hippocampal acetylcholine concentration, the time-courses of the two effects were not identical.

The different time-course raises the possibility that the two phenomena may be dissociated. This possibility has been suggested by Lichtman and Martin (1996) who found that Δ^9-THC-induced working memory impairment was not reversed by physostigmine, as would have been expected if acetylcholine reduction was responsible for Δ^9-THC amnesic effect. Moreover, several studies have shown that cannabinoid inhibition of adenylate cyclase produces a modulation of conductance at a voltage-dependent K^+ channel in the hippocampus without cholinergic neuronal mediation (Childers and Deadwyler, 1996; Deadwyler et al., 1995), indicating that cannabinoids may affect hippocampal function in an acetylcholine-independent manner. The different time courses of Δ^9-THC effects suggest at least four alternative hypotheses. The first possibility is that Δ^9-THC effects on hippocampal extracellular acetylcholine concentration are not involved in the reduction of correct alternation tasks in the T-maze. The second is that the extracellular acetylcholine concentrations measured by means of microdialysis technique do not accurately reflect the changes in acetylcholine levels induced by Δ^9-THC at hippocampal synapses. The third is that the discrepancy between the inhibition of acetylcholine release and the reduction of correct alternation tasks in the T-maze is the result of the dialysis probe placement in a hippocampal area not coincident with the CA3 region controlling memory processes. The fourth is that the inhibition of hippocampal acetylcholine concentration and the reduction of correct alternation tasks in the T-maze are not directly correlated but may be controlled by a third neurochemical system (Nava et al., 2000). A large body of evidence is in favour of this last hypothesis (Nava et al., 2000). On the other hand, acetylcholine is only one of the neurotransmitters involved in the amnesiac effects induced by cannabinoids.

The second and major outcome of this study is that no tolerance to the negative Δ^9-THC-effects developed after chronic drug treatment. The molecular mechanisms underlying the lack of tolerance both on the reduction of extracellular acetylcholine concentration and correct alternation tasks in the T-maze are still unknown. Tolerance to cannabinoids appears to result from both pharmacokinetic and pharmacodynamic changes. Some evidence suggests that chronic treatment with the synthetic cannabinoid agonist CP 55,940 increases the activity of the microsomal cytochrome P450 oxidase system (Costa et al., 1996). On the other hand, other studies have found that a prolonged exposure to cannabinoids induce a brain reduction (Breivogel et al., 1999; Fan et al., 1996; Oviedo et al., 1993; Rodriguez de Fonseca et al., 1992) and a desensitization (Rodriguez de Fonseca et al., 1994; Sim et al., 1996) of CB_1 cannabinoid receptors. However, irrespective of the mechanisms involved, due to the fact that SR 141716A reverses the negative effects induced by chronic Δ^9-THC treatment (data not shown) we cannot exclude a direct, although still unknown, role of CB_1 cannabinoid receptors in the control of the detrimental effects induced by a chronic treatment.

A large body of evidence indicates an involvement of the dopaminergic system in the cognitive deficit induced by cannabinoids. In fact, Jentsch et al. (1998) have found that repeated and intermittent exposure to cannabinoids elicits a reduction in prefrontal cortical dopamine transmission which may account in part for memory deficits induced by Δ^9-THC. Indeed, our recent results have shown that S(±)-sulpiride, a D_2 dopamine receptor antagonist, reverses the negative effects induced by Δ^9-THC (Nava et al., 2000). Furthermore, a recent study by Wu and French (2000) showed that during chronic treatment with Δ^9-THC, the neurons of substantia nigra pars compacta (SNpc) developed tolerance to the effects of Δ^9-THC, while the neurons of midbrain ventral tegmental area (VTA) exhibited continued increases of firing rate when challenged repeatedly with Δ^9-THC. Interestingly, these authors have demonstrated that no tolerance develops after chronic Δ^9-THC treatment to the increase of firing of the meso-limbic and -cortical dopaminergic neurons (Wu and French, 2000). Since dopamine release in the limbic and cortical areas is the neurochemical substrate underlying the rewarding properties of different drugs of abuse, including marijuana (Gardner and Lowinson, 1991), the lack of tolerance to the increase of firing rate in VTA neurons could represent a possible mechanism by which tolerance does not develop to the euphorigenic effects induced by marijuana (Dewey, 1986; Perez-Reyes et al., 1991). On the other hand, during a chronic Δ^9-THC treatment, the hyperactivity of dopaminergic system in the limbic and cortical areas could be responsible of the persistent memory deficits occurring during a chronic Δ^9-THC treatment or in chronic marijuana abusers (Chait and Perry, 1994). The mechanisms by which chronic Δ^9-THC treatment does not induce tolerance in VTA neurons remains unknown (Wu and French, 2000). Others experimental data on animals have shown that a heavy (10 mg/kg) and chronic Δ^9-THC treatment (until 35 days) can induce, both in rats (Deadwyler et al., 1995) and in monkeys (Branch et al., 1980), tolerance to the memory disruptive effects induced by the drug. Considering the above studies, we may also suggest that a long term and heavy Δ^9-THC treatment may be sufficient

to develop tolerance both to the reduction of acetylcholine release and correct alternation tasks in the T-maze. The previous studies also confirm, together with our evidence, that a heavy Δ^9-THC and long-term treatment does not necessarily induce persistent and irreversible impairment of cognition. In fact, in our experiments, the possibility that some residual Δ^9-THC levels at 12 h post-administration were substantially present may be ruled out since neither reduction of correct alternation tasks in the T-maze nor acetylcholine changes were present in chronically treated rats when tested 12 h after the last chronic Δ^9-THC dose. However, there is still some doubt as to whether a heavy use of marijuana may induce persistent cognitive deficits. A recent work has indicated that heavy use of marijuana is associated with residual neuropsychological effects even after a day of abstinence from the drug (Pope et al., 1995). However, the question if chronic marijuana use induces persistent cognitive deficits remains unsolved because we do not know whether impairment is due to a residual effect of the drug in the brain, to withdrawal from the drug, or to a neurotoxic effect of marijuana (Pope et al., 1995).

Irrespective of the role of acetylcholine in Δ^9-THC amnesiac effects, the lack of tolerance to the memory deficit is in agreement with the clinical observation that cognitive impairment does not wean in chronic marijuana users (Chait and Perry, 1994). More complex is the question regarding the cognitive performance over the time in chronic marijuana abusers. A recent work suggests that the memory deficit are worse in older long-term *cannabis* users than in younger users or in *cannabis* non-users (Fletcher et al., 1996). Neuroimaging studies have shown any cerebral anatomical abnormalities in chronic *cannabis* users (Co et al., 1997; Kuehnle et al., 1997) although Volkow et al. (1996) have demonstrated that in these subjects there is an alteration of cerebral glucose metabolism. However, our findings showing that the negative effects of Δ^9-THC are not more present 12 h after the last acute or chronic drug treatment supports the view that Δ^9-THC could not produce persistent damages in short-term memory processes (Stiglick and Kalant, 1985), although the chronic *cannabis* abuse may induce a reduction of several cognitive functions (Pope and Yurgelun-Todd, 1996; Solowij, 1995).

The cognitive deficit induced by Δ^9-THC remains the most poorly understood aspect of the neurobiology of cannabinoids. In fact, the molecular mechanisms underlying the lack of tolerance to the cognitive deficit induced by a chronic Δ^9-THC treatment, as well as, the neurochemical basis of the reduction of cognitive functions are not yet well known but they are under investigation in our laboratories.

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References

Aigner, T.G., 1995. Pharmacology of memory: cholinergic–glutamatergic interactions. Current Opinion in Neurobiology 5, 155–160.

Branch, M.N., Dearing, M.E., Lee, D.M., 1980. Acute and chronic effects of delta9-tetrahydrocannabinol on complex behaviour of squirrel monkeys. Psychopharmacology 71, 247–256.

Breivogel, C.S., Childers, S.R., Deadwyler, S.A., Hampson, R.E., Vogt, L.J., Sim-Selley, C.J., 1999. Chronic Δ^9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. Journal of Neuroscience 73, 2447–2459.

Brodkin, J., Moerschbaecher, J.M., 1997. SR 141716A antagonizes the disruptive effects of canabinoid ligands on learning in rats. Journal of Pharmacology and Experimental Therapeutics 282, 1526–1532.

Carta, G., Nava, F., Gessa, G.L., 1998. Inhibition of hippocampal acetylcholine release after acute and repeated Δ^9-tetrahydrocannabinol in rats. Brain Research 809, 1–4.

Chait, L.D., Perry, J.L., 1994. Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. Psychopharmacology 115, 340–349.

Chen, J., Paredes, W., Lowinson, J.H., Gardner, E.L., 1990. Delta9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. European Journal of Pharmacology 190, 259–262.

Childers, S.R., Deadwyler, S.A., 1996. Role of cyclic AMP in the actions of cannabinoid receptors. Biochemical Pharmacology 52, 819–827.

Co, B.T., Goodwin, D.W., Gado, M., Mikhael, M., Hill, S.Y., 1997. Absence of cerebral atrophy in chronic cannabis users by computerized transaxial tomography. Journal of the American Medical Association 237, 1229–1230.

Collins, D.R., Pertwee, R.G., Davies, S.N., 1995. Prevention by the cannabinoid antagonist, SR 141716A, of cannabinoid-mediated blockade of long-term potentation in the rat hippocampal slice. British Journal of Pharmacology 115, 869–870.

Costa, B., Parolaro, D., Colleoni, M., 1996. Chronic cannabinoid, CP-55,940, administration alters biotransformation in rat. European Journal of Pharmacology 313, 17–24.

Damsa, G., Westernik, B.H.C., 1991. A microdialysis and automated on-line analysis approach to study central cholinergic transmission in vivo. In: Robinson, T.E., Justice, J.B. (Eds.), Microdialysis in the Neuroscience. Elsevier, Amsterdam, pp. 237–252.

Deadwyler, S.A., Hampson, R.E., Mu, J., Whyte, A., Childers, S., 1995. Cannabinoids modulate voltage sensitive potassium A — current in hippocampal neurons via a cAMP-dependent process. Journal of Pharmacology and Experimental Therapeutics 273, 734–743.

Dewey, W.L., 1986. Cannabinoid pharmacology. Pharmacological Review 38, 151–178.

Diana, M., Melis, M., Gessa, G.L., 1998. Increase in meso-prefrontal dopaminergic activity after stimulation of CB_1 receptors by cannabinoids. European Journal of Neuroscience 10, 2825–2830.

Evans, S., 1992. Effects of drug abuse on acquisition of behavioural chain in squirrel monkeys. Psychopharmacology 107, 55–60.

Fan, F., Tao, Q., Abood, M.E., Martin, B.R., 1996. Cannabinoid receptors down-regulation without alteration of the inhibitory effect of CP 55,940 on adenyl cyclase in the cerebellum of CP 55,940 tolerant mice. Brain Research 706, 13–20.

Fletcher, J.M., Page, J.B., Francis, D.J., Copeland, K., Naus, M.J., Davis, C.M., Morris, R., Krausskopf, D., Satz, P., 1996. Cognitive correlates of long-term cannabis use in Costa Rican man. Archives of General Psychiatry 53, 1051–1057.

Gardner, E.L., Lowinson, J.H., 1991. Marijuana's interaction with brain reward system: update 1991. Pharmacological and Biochemical Behaviour 40, 571–580.

Gessa, G.L., Mascia, M.S., Casu, M.A., Carta, G., 1997. Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR 141716A. European Journal of Pharmacology 327, R1–R2.

Gessa, G.L., Casu, M.A., Carta, G., Mascia, M.S., 1998a. Cannabinoids decrease acetylcholine release in the medial prefrontal cortex and hippocampus, reversal by SR 141716A. European Journal of Pharmacology 355, 119–124.

Gessa, G.L., Melis, M., Muntoni, A.L., Diana, M., 1998b. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB_1 receptors. European Journal of Pharmacology 341, 39–44.

Gifford, A.N., Ashby, C.R. Jr., 1996. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid receptor antagonist, SR 141716A. Journal of Pharmacology and Experimental Therapeutics 277, 1431–1436.

Gifford, A.N., Bruneus, M., Gatley, S.J., Volkow, N.D., 2000. Cannabinoid receptor-mediated inhibition of acetylcholine release from hippocampal and cortical synaptosomes. British Journal of Pharmacology 131, 645–650.

Hall, W., Solowij, N., 1998. Adverse effects of cannabis. Lancet 352, 1611–1616.

Heishman, S.J., Arastesh, K., Stitzer, M.L., 1997. Comparative effects of alcohol and marijuana on mood, memory and performance. Pharmacology Biochemistry and Behavior 58, 93–101.

Heyser, C.J., Hampson, R.E., Deadwyler, A., 1993. Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alternations in short-term memory associated with changes in task specific firing of hippocampal cells. Journal of Pharmacology and Experimental Therapeutics 264, 294–307.

Herkenham, M., 1995, Localization of cannabinoid receptors in brain and periphery. In: Pertwee, R.G. (Ed.), Cannabinoid Receptors. Academic Press, New York, pp. 145–166.

Hollister, L.E., 1986. Health aspects of cannabis. Pharmacological Review 38, 1–20.

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 rats: blockade of dopaminergic effects with HA 966. Neuropsychopharmacology 16, 426–432.

Jentsch, J.D., Verrico, C.D., Le, D., Roth, R.H., 1998. Repeated exposure to delta-9-tetrahydrocannabinol reduces prefrontal cortical dopamine metabolism in the rat. Neuroscience Letters 246, 169–172.

Kuehnle, J., Mendelson, J.H., Davis, K.R., New, P.F., 1997. Computed tomographic examination of heavy marihuana smokers. Journal of the American Medical Association 237, 1231–1232.

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

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

Mallet, P.E., Beninger, R.J., 1998. The cannabinoid CB_1 receptor antagonist SR 141716A attenuates the memory impairment produced by delta-9-tetrahydrocannabinol or anandamide. Psychopharmacology 140, 11–19.

Miller, L.L., Branconnier, R.L., 1983. Cannabis: effects on memory and the cholinergic limbic system. Psychological Bulletin 93, 441–456.

Nava, F., Carta, G., Battasi, A.M., Gessa, G.L., 2000. D_2 dopamine receptors enable Δ^9-tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. British Journal of Pharmacology 130, 1201–1210.

Oviedo, A., Glowa, J., Herkenham, M., 1993. Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. Brain Research 618, 293–302.

Paxinos, G., Watson, C., 1986, The Rat Brain in Stereotaxic Coordinates. Academic Press, London.

Perez-Reyes, M., White, W.R., McDonald, S.A., Hichs, R.E., Jeffcoat, A.R., Cook, C.E., 1991. The pharmacological effects of dialy marijuana studing in humans. Pharmacology Biochemistry and Behavior 40, 691–694.

Perry, E., Walker, M., Grace, J., Perry, R., 1998. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends in Neuroscience 22, 273–280.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacology and Therapeutics 74, 129–180.

Pope, H.G. Jr., Gruber, A.J., Yurgelun-Todd, D., 1995. The residual neuropsychological effects of cannabis: the current status of research. Drug and Alcohol Dependence 38, 25–34.

Pope, H.G. Jr., Yurgelun-Todd, D., 1996. The residual cognitive effects of heavy marijuana use in college students. Journal of the American Medical Association 275, 521–527.

Reibaud, M., Obinu, M.C., Ledent, C., Parmentier, M., Bohme, G.A., Imperato, A., 1999. Enhancement of memory in cannabinoid CB_1 receptor knock-out mice. European Journal of Pharmacology 379, R1–R2.

Rodriguez De Fonseca, F., Fernandez-Ruiz, J.J., Murphy, L.L., Cebeira, M., Steger, R.W., Bartke, A., Ramos, J.A., 1992. Acute effects on delta9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. Pharmacology Biochemistry and Behavior 42, 269–275.

Rodriguez De Fonseca, F., Gorriti, M.A., Fernandez-Ruiz, J.J., Palomo, T., Ramos, J.A., 1994. Down-regulation of rat brain cannabinoid binding sites after chronic delta-9-tetrahydrocannabinol. Pharmacology Biochemistry and Behavior 47, 33–40.

Shepard, P.D., Coonnelly, S.T., Lehmann, H., Grobaski, K.C., 1995. Effects of the enantiomers of (±)-HA-966 on dopamine neurons: an electrophysiological study of a chiral molecule. European Journal of Pharmacology 285, 79–88.

Sim, L.J., Hampson, R.E., Deadwyler, S.A., Childers, S.R., 1996. Effects of chronic treatment with Δ^9-tetrahydrocannabinol on cannabinoid-stimulated [^{35}S] GTPγ S autoradiography in rat brain. Journal of Neuroscience 16, 8057–8066.

Solowij, N., 1995. Do cognitive impairments recover following cessation of cannabis use? Life Science 56, 2119–2126.

Stiglick, A., Kalant, H., 1985. Residual effects of chronic cannabis treatment on behaviour in mature rats. Psychopharmacology 85, 436–439.

Terranova, J.P., Storme, J.J., Lafon, N., Perio, A., Rinaldi-Carmona, M., Le Fur, G., Soubrie, P., 1996. Improvement of memory in rodents by the selective CB_1 cannabinoid receptor antagonist, SR 141716A. Psychopharmacology 126, 165–172.

Vizi, E.S., Kiss, J.P., 1998. Neurochemistry and pharmacology of the major hippocampal transmitter systems: synaptic and non-synaptic interactions. Hippocampus 8, 566–607.

Volkow, N.D., Gillepie, H., Mullani, N., Tancredi, L., Grant, C., Valentine, A., Hollister, L., 1996. Brain glucose metabolism in chronic marijuana users at baseline and drug marijuana intoxication. Psychiatry Research 67, 29–38.

Wu, X., French, E.D., 2000. Effects of chronic Δ^9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. Neuropharmacology 39, 391–398.