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Brain Research 809 (1998) 1–4

BRAIN RESEARCH

Research report

Inhibition of hippocampal acetylcholine release after acute and repeated Δ^9-tetrahydrocannabinol in rats

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Accepted 14 July 1998

Abstract

The effects of acute and repeated administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive principle of marijuana, on acetylcholine release in the hippocampus was studied in freely moving rats by microdialysis. The acute intraperitoneal (i.p.) administration of Δ^9-THC at the doses of 2.5 and 5 mg/kg reduced acetylcholine release by about 25% and 45%, respectively. A dose of 7.5 mg/kg produced no further reduction. Δ^9-THC effects were antagonized by the cannabinoid CB_1 antagonist SR141716A at the i.p. dose of 1 mg/kg, per se ineffective in modifying acetylcholine concentrations. After a repeated exposure (twice daily for up to seven days) to Δ^9-THC (7.5 mg/kg, i.p.) or vehicle (0.3 ml/kg, i.p.), the inhibitory effect of Δ^9-THC (2.5 and 5 mg/kg, i.p) on acetylcholine release was not reduced. The results confirm previous observations that cannabinoids inhibit acetylcholine release through cannabinoid CB_1 receptors, and indicate that no tolerance to this effects develops after a repeated Δ^9-THC administration.

Keywords: Δ^9-Tetrahydrocannabinol; CB_1 receptor; Hippocampus; Microdialysis; Acetylcholine; SR141716A

1. Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the psychoactive principle of marijuana, is known to produce impairment in learning and memory both in humans and laboratory animals [3,4,8,13,14,16,18]. Impairment of memory processes is also produced by the synthetic cannabinoid CB_1 agonists, WIN 55,212-2 {*R*-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinylmethyl] pyrol [1,2,3-*de*]1,4-benzoxazin-6-yl) (L-naphthalenyl) methanone monomethanesulfonate} and CP 55,940 {[1a,2-(r)-5-(1,1-dimethylpheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-phenol} [18,24] and by the endogenous cannabinoid agonists, anandamide [20] and 2-AG (*sn*-2 arachidonylglycerol) [23]. The negative effects of the cannabinoid agonists are blocked by the cannabinoid CB_1 antagonist SR 141716A, a compound that is per se able to improve memory processes in rodents [3,24]. These results indicate that the impairment in memory by Δ^9-THC is mediated via CB_1 receptors [19,24] and also suggest that endogenous cannabinoids play a role in cognitive processes [20,23]. Moreover, experimental evidence suggests that inhibition of cholinergic neurotransmission in the hippocampus plays an important role in cognitive alterations produced by cannabinoids [21]. Thus, cannabinoid agonists impair working memory after intrahippocampal administration [18] and inhibit long-term potentiation in hippocampal slices [5], a classic electrophysiological model for the study of learning and memory processes [1]. In addition, cannabinoid agonists reduce acetylcholine output in the hippocampus in freely moving rats [10] and [^{14}C] acetylcholine release in hippocampal slices [11,12].

The present study, using microdialysis techniques in freely moving rats, was carried out to determine whether the suppressant effect of Δ^9-THC on hippocampal acetylcholine release would be modified by a repeated exposure to the drug. This problem is clinically relevant since

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0006-8993/98/$ - see front matter
PII: S0006-8993(98)00738-0

chronic marijuana use has been claimed to produce persistent adverse effects on cognition [4,16].

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (225–250 g; Charles River, Calco, Lecco, Italy) were housed in groups of three per cage for at least ten days before use. Food and water were freely available and animals were maintained under an artificial 12-h/12-h light/dark cycle (lights were on from 0700 to 1900). Experiments were carried out between 0800 and 1700 h.

2.2. Microdialysis implantation and experimental procedure

Rats were anesthetised with equitensin (4 mg/kg, i.p.) and dialysis tubes (AN 69-HF, with a wet fiber outer diameter of 320 μm; Hospal-Dasco, Bologna, Italy) were implanted at the level of the dorsal hippocampus ($A = -3.2$ from the bregma and $V = -3.6$ from the skull), according to the atlas by Paxinos and Watson [22]. The localisation of the dialysis probe was verified at the end of the experiments [17].

Experiments started 24 h after surgery.

The Ringer solution containing 3 mM KCl, 125 mM NaCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23 mM $NaHCO_3$, 1.5 mM potassium phosphate buffer (pH 7.3), and 0.1 μM neostigmine bromide was pumped through the dialysis probe at a constant rate of 2 μl/min. Samples were collected every 20 min, corresponding to a volume of 40 μl, and were injected in a high-performance liquid chromatography (HPLC) with electrochemical detection according to the techniques described by Damsma and Westerink [6]. The detection limit for acetylcholine was 0.05 fmol per 1 μl of sample.

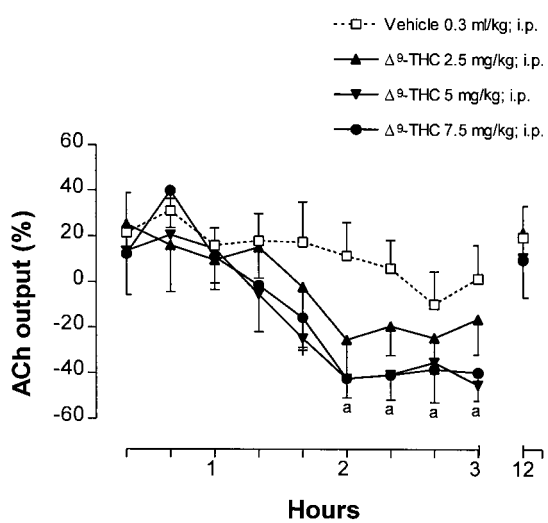

Fig. 1. Effect of Δ^9-THC (2.5, 5 and 7.5 mg/kg, i.p.) on extracelluar acetylcholine release in the hippocampus. At the time 0 all groups were injected with Δ^9-THC or vehicle (0.3 ml/kg, i.p.) Baseline acetylcholine concentrations were 1.28 ± 0.32, 1.08 ± 1.12 and 1.42 ± 0.42 fmol/1 μl for the Δ^9-THC dose of 2.5, 5 and 7.5 mg/kg, respectively. Results (means ± S.E.M.; $n = 5$) are expressed as a percentage of basal values. (a) $P < 0.05$ vs. the previous doses measured at 80 min from Δ^9-THC administration (Student–Newman–Keuls test).

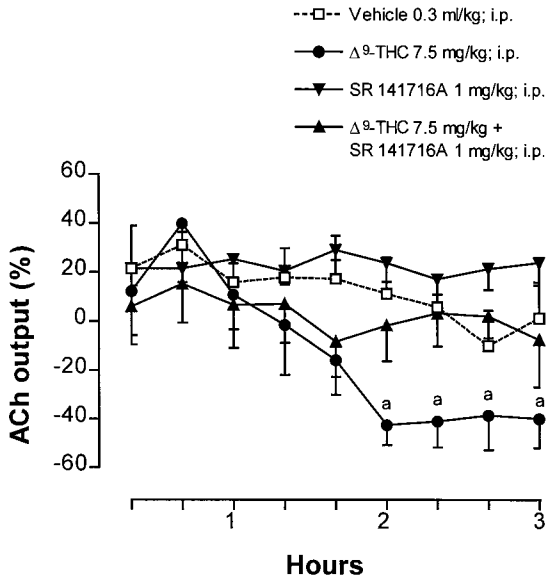

Fig. 2. Effect of Δ^9-THC (5 mg/kg, i.p.), SR 141716A (1 mg/kg, i.p.), and Δ^9-THC (5 mg/kg, i.p.) + SR 141716A (1 mg/kg, i.p.) on extracellular acetylcholine release in the hippocampus. Baseline acetylcholine concentrations were 1.17 ± 1.10, 1.25 ± 0.5 and 1.42 ± 2.1 fmol/1 μl for the Δ^9-THC, SR 141716A, and Δ^9-THC + SR 141716A, respectively. Results (means ± S.E.M.; $n = 5$) are expressed as a percentage of basal values. (a) $P < 0.05$ vs. the previous doses measured at 80 min from Δ^9-THC administration (Student–Newman–Keuls test).

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% and all subsequent post-treatment values are expressed as mean (+S.E.M.) percent variation of basal values.

The basal extracellular concentration of acetylcholine in the hippocampus, at 24 h after surgery, was 1.23 ± 0.18 fmol/μl ($n = 5$).

2.3. Drugs

Δ^9-THC was purchased from RBI (Amersham, Life Science, MA, USA), while SR 141716A was a gift from Sanofi Recherche (Montpellier, France). Drugs were dissolved in Tween 80 (Merk, Milan, Italy) and then in a NaCl solution 0.9%.

2.4. Statistical analysis

Between-group comparisons were assessed by a two-way ANOVA for repeated measures, factors being treat-

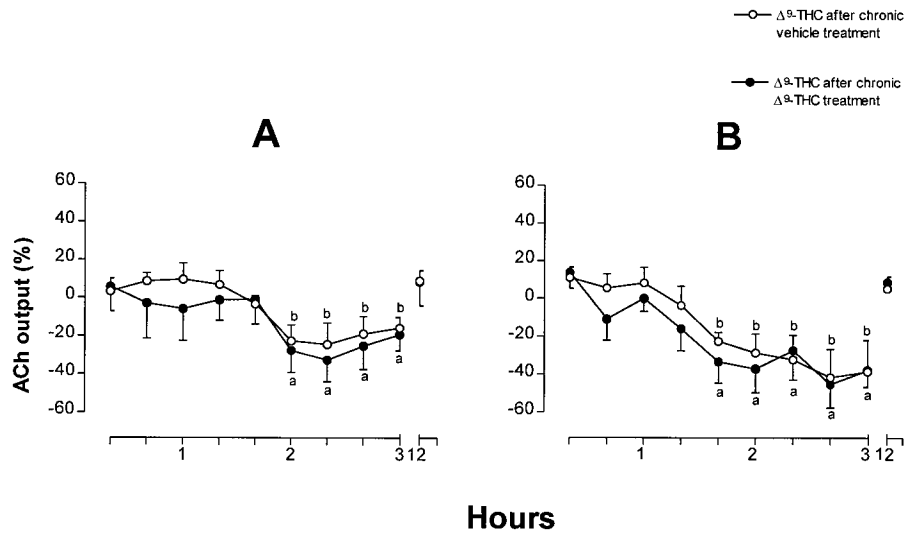

Fig. 3. Effect of Δ^9-THC, 2.5 (A) and 5 mg/kg (B) on extracellular acetylcholine release in the hippocampus after chronic treatment (twice daily for seven days) of Δ^9-THC (7.5 mg/kg, i.p.) or vehicle (0.3 ml/kg). The Δ^9-THC challenge was given 12 h after the last chronic treatment. Baseline acetylcholine concentrations in A were 1.14 ± 0.6 and 1.45 ± 0.34 fmol/1 μl and in B 1.10 ± 1.2 and 1.07 ± 0.25 fmol/1 μl for Δ^9-THC and vehicle, respectively. Results (means ± S.E.M.; $n = 5$) are expressed as a percentage of basal values. (a, b) $P < 0.05$ vs. the previous doses measured at 80 min from Δ^9-THC administration (Student–Newman–Keuls test).

ment and time points. Post-hoc analysis was performed using the Student–Newman–Keuls test.

3. Results

3.1. Effects of a single Δ^9-THC administration on hippocampal acetylcholine output

As shown in Fig. 1 the administration of Δ^9-THC at the dose of 2.5, and 5 mg/kg inhibited acetylcholine release by about 25% (ANOVA main effect $F_{1,8} = 3.81$; post-hoc $P > 0.05$; ANOVA main effect of repeated measures $F_{8,36} = 1.37$; post-hoc $P > 0.05$) and 45% (ANOVA main effect $F_{1,8} = 12.17$; post-hoc $P < 0.05$; ANOVA main effect of repeated measures $F_{8,36} = 4.96$; post-hoc $P < 0.05$), respectively (Fig. 1). A higher dose of 7.5 mg/kg produced no further reduction (ANOVA main effect $F_{1,8} = 5.55$; post-hoc $P < 0.05$; ANOVA main effect of repeated measures $F_{8,36} = 4.96$; post-hoc $P < 0.05$) (Fig. 1). Maximal inhibition of acetylcholine output occurred at 2 h after treatment and persisted longer than 3 h. Acetylcholine concentration returned to baseline within 12 h after Δ^9-THC treatment (Fig. 1).

The inhibitory effect was prevented by the specific cannabinoid CB_1 receptors antagonist SR141716A at the dose of 1 mg/kg (ANOVA main effect $F_{1,8} = 4.37$; post-hoc $P > 0.05$; ANOVA main effect of repeated measures $F_{8,36} = 0.24$; post-hoc $P > 0.05$), which per se did not modify acetylcholine concentration (ANOVA main effect $F_{1,8} = 6.03$; post-hoc $P > 0.05$; ANOVA main effect of repeated measures $F_{8,36} = 0.22$; post-hoc $P > 0.05$) (Fig. 2).

3.2. Effects of a repeated Δ^9-THC administration on hippocampal acetylcholine output

After a repeated vehicle treatment (0.3 ml/kg, i.p.; twice daily for seven days) a single dose of Δ^9-THC (7.5 mg/kg, i.p.), given 12 h after the last chronic treatment, produced the same inhibitory effect observed after a single Δ^9-THC administration (ANOVA main effect of repeated measures $F_{8,36} = 3.82$; post-hoc $P < 0.05$) (Fig. 3). Indeed, a single dose of Δ^9-THC (2.5 and 5 mg/kg), given 12 h after the chronic Δ^9-THC treatment (7.5 mg/kg, i.p. twice daily for seven days), produced the same degree of inhibition in rats that received chronically vehicle and then a single dose of Δ^9-THC (2.5 and 5 mg/kg, i.p.) (ANOVA main effect between treatment $F_{1,8}$ Δ^9-THC 2.5 mg/kg = 0.8; post-hoc $P > 0.05$; $F_{1,8}$ Δ^9-THC 5 mg/kg = 0.03; post-hoc $P > 0.05$; ANOVA main effect of repeated measures $F_{8,36}$ Δ^9-THC 2.5 mg/kg = 4.28.; post-hoc $P < 0.05$; $F_{8,36}$ Δ^9-THC 5 mg/kg = 15.40; post-hoc $P < 0.05$) (Fig. 3).

The time-course of acetylcholine decline in chronically Δ^9-THC treated rats was the same as in vehicle treated ones: the inhibition peaked at 2 h persisted for it last 3 h and was gone by 12 h (Fig. 3).

4. Discussion

This study confirms previous observations showing that different cannabinoid agonists inhibit acetylcholine release in the hippocampus in vivo [10] as well as in hippocampal slices [5,11] and that this effect is prevented by the CB_1 receptor antagonist SR141716A. Moreover, this study

shows that the inhibitory effects of Δ^9-THC develops after a long delay and is a lasting response: acetylcholine concentrations were reduced about 2 h after treatment and the reduction persisted for over 3 h. The reason for the delayed inhibitory effect on the acetylcholine release is not clear. The delay might indicate the presence of an active metabolite in brain. This possibility is suggested by the fact that a more rapid decrease in acetylcholine output was observed after the administration of the synthetic cannabinoid agonists WIN 55,212-2 and CP 55,940 (paper in preparation). However, since Δ^9-THC interacts with CB_1 receptors controlling different neuronal circuits the action of its systemic administration is not easy predictable. Local perfusion of Δ^9-THC and other cannabinoid agonists in the hippocampus will help in clarifying this issue. These experiments might also clarify whether the effect of cannabinoids are mediated by CB_1 receptors localized in the hippocampus itself [15,25] or whether systemic cannabinoids inhibit septo-hippocampal cholinergic neurons by acting at the somatodendritic receptors in the septum [7].

An important outcome from the present study is that no tolerance to the inhibitory effects develops after repeated treatment. Since the inhibition of acetylcholine output might represent the neurochemical substrate of the memory impairment [2] caused by cannabinoids, the lack of tolerance to the inhibitory effect on acetylcholine release is in agreement with the lack tolerance to the memory impairment caused by cannabinoids in animals [9] and with the clinical observation that impairment in learning and memory does not wean in chronic marijuana users [4,14].

Δ^9-THC effects on acetylcholine release were produced by doses within a range believed to be pharmacologically relevant to human marijuana users, therefore our research suggest that the reduction of hippocampal acetylcholine release might play a role in the negative effects of marijuana on cognitive processes.

Further studies are in progress in our laboratory to correlate the cannabinoid inhibition acetylcholine output in hippocampus and operant learning and memory processes in rats.

References

[1] T.V.P. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[2] A. Blokland, Acetylcholine: a neurotransmitter for learning and memory?, Brain Res. Rev. 21 (1995) 285–300.
[3] J. Brodkin, J.M. Moerschbaecher, SR141716A antagonizes the disruptive effects of cannabinoid ligands on learning in rats, JPET 282 (1997) 1526–1532.
[4] L.D. Chait, J.L. Perry, Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance, Psychopharmacology 115 (1994) 340–349.
[5] D.R. Collins, R.G. Pertwee, S.N. Davies, Prevention by the cannabinoid antagonist SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice, Br. J. Pharmacol. 115 (1995) 869–870.
[6] G. Damsma, B.H.C. Westerink, A microdialysis and automated on-line analysis approach to study central cholinergic transmission in vivo, in: T.E. Robinson, J.B. Justice (Eds.), Microdialysis in the Neuroscience, Elsevier, Amsterdam, 1991, pp. 237–252.
[7] J.C. Day, H.C. Fibiger, Dopaminergic regulation of septohippocampal cholinergic neurons, J. Neurochem. 63 (1994) 2086–2092.
[8] S. Evans, Effects of drug abuse on acquisition of behavioural chain in squirrel monkeys, Psychopharmacology 107 (1992) 55–60.
[9] D.P. Ferraro, D.M. Grilly, Lack of tolerance to Δ^9-tetrahydrocannabinol in chimpanzees, Science 179 (1973) 490–492.
[10] G.L. Gessa, M.S. Mascia, M.A. Casu, G. Carta, Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR 141716A, Eur. J. Pharmacol. 327 (1997) R1–R2.
[11] A.N. Gifford, C.R. Ashby Jr., Electrically evoked acetylcholine release from hippocampal slice is inhibited by the cannabinoid receptor agonists, WIN 55212-2, and is potentiated by the cannabinoid agonist, SR 141716A, JPET 277 (1996) 1431–1436.
[12] A.N. Gifford, L. Samiian, S.J. Gatley, C.R. Ashby Jr., Examination of the effect of the cannabinoid receptor agonist, CP 55,940 on electrically evoked transmitter release from rat brain slices, Eur. J. Pharmacol. 324 (1997) 187–192.
[13] M. Haney, S.D. Comer, A. Ward, R.W. Foltin, M.W. Fischman, Factors influencing marijuana self-administration by humans, Behav. Pharmacol. 8 (1997) 101–112.
[14] S.J. Heishman, K. Arasteh, M.L. Stitzer, Comparative effects of alcohol and marijuana on mood, memory and performance, Pharmacol. Biochem. Behav. 58 (1997) 93–101.
[15] M. Herkenham, A.B. Lynn, M.R. Jhonson, L.S. Melvin, B.R. De Costa, K.C. Rice, Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study, J. Neurosci. 11 (1991) 563–583.
[16] C.J. Heyser, R.E. Hampson, S.A. Deadwyler, 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, JPET 264 (1993) 294–307.
[17] A. Imperato, L. Angelucci, P. Casolini, A. Zocchi, S. Puglisi-Allegra, Repeated stressful experiences differently effect limbic dopamine release during and following stress, Brain Res. 577 (1992) 194–199.
[18] A.H. Lichtman, K.R. Dimen, B.R. Martin, Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats, Psychopharmacology 119 (1995) 282–290.
[19] A.H. Lichtman, B.R. Martin, Δ^9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism, Psychopharmacology 126 (1996) 125–131.
[20] P.E. Mallet, R.J. Benninger, The endogenous cannabinoid receptor agonist anandamide impairs memory in rats, Behav. Pharmacol. 7 (1996) 276–284.
[21] L.L. Miller, R.J. Bracconnier, Cannabis: effects on memory and the cholinergic limbic system, Psychol. Bull. 93 (1983) 441–456.
[22] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, London, 1986.
[23] N. Stella, P. Schweitzer, D. Piomelli, A second endogenous cannabinoid that modulates long-term potentiation, Nature 388 (1997) 773–778.
[24] J.P. Terranova, J.J. Storme, N. Lafon, A. Pério, M. Rinaldi-Carmona, G. Le Fur, P. Soubrié, Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716, Psychopharmacology 126 (1996) 165–172.
[25] B.F. Thomas, X. Wei, B.R. Martin, Characterization and autoradiographic localization of the cannabinoids binding site in rat brain using [^{3}H]11-OH-D9-THC-DMH, JPET 263 (1992) 1383–1390.