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Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A

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Abstract

The effect of Δ^9-tetrahydrocannabinol, the psychoactive principle of marijuana, and {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinylmethyl]pyrol[1,2,3-de-]-1,4-benzoxazin-6y)(1-naphthalenyl)methanone monomethanesulfonate} (WIN 55,212-2), a synthetic cannabinoid receptor agonist, on the acetylcholine output in the medial–prefrontal cortex and hippocampus was studied by microdialysis in freely moving rats. The administration of Δ^9-tetrahydrocannabinol (1 and 5 mg/kg i.p.) and WIN 55,212-2 (5 and 10 mg/kg i.p.) produced a long lasting inhibition of acetylcholine release in both areas. The inhibitory effect of Δ^9-tetrahydrocannabinol and WIN 55,212-2 was suppressed in both areas by the specific cannabinoid CB_1 receptor antagonist, {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3carboxamide}HCl (SR 141716A), at the dose of 0.1 mg/kg i.p., per se ineffective to modify basal acetylcholine release. Most interestingly, SR 141716A alone at higher doses increased acetylcholine release both in the medial–prefrontal cortex (3 mg/kg i.p.) and hippocampus (1 and 3 mg/kg i.p.), suggesting that acetylcholine output is tonically inhibited by endogenous cannabinoids. Since the inhibitory effect of Δ^9-tetrahydrocannabinol is produced by doses within those relevant to human use of marijuana, our results suggest that the negative effects of the latter on cognitive processes may be explained by its ability to reduce acetylcholine release in the medial–prefrontal cortex and hippocampus. Conversely, cannabinoid receptor antagonists may offer potential treatments for cognitive deficits.

Keywords: Cortex, medial–prefrontal; Hippocampus; Acetylcholine; Microdialysis; Cannabinoid receptor agonist; SR 141716A

1. Introduction

Cannabinoids have long been known to impair learning and memory in a variety of tasks in rodents, non-human primates and humans (Compton et al., 1996). In addition to Δ^9-tetrahydrocannabinol, the psychoactive principle of marijuana, structurally distinct synthetic cannabinoid receptor agonists, WIN 55,212-2, CP 55,940, *R*-methanandamide and the endogenous cannabinoid receptor agonist, anandamide, were also found to impair working memory in rats (Lichtman et al., 1995; Brodkin and Moerschbaecher, 1997; Mallet and Beninger, 1996).

Recent studies in vitro have shown that electrically-evoked [^{14}C] acetylcholine release from hippocampal and striatal slices is inhibited by the cannabinoid receptor agonists CP 55,940 and WIN 55,212-2 and is potentiated by the cannabinoid receptor antagonist SR 141716A (Gifford and Ashby, 1996; Gifford et al., 1997).

On the other hand, several lines of evidence indicate that acetylcholine in the medial–prefrontal cortex and hippocampus is involved in cognition-related processes such as arousal, attention, learning and memory (Delacour et al., 1990; Penttila et al., 1985; Riekkinen et al., 1991; Simon and Emmons, 1956). Accordingly, administration of muscarinic receptor antagonists and neurotoxin-induced lesions of cortical or hippocampal cholinergic neurons cause impairments in performance of learning and memory tasks (Fibiger, 1991).

More recently, using brain microdialysis in freely moving rats we have shown that WIN 55,212-2 and CP 55,940 inhibit acetylcholine release in the rat hippocampus, this effect being reversed by the specific cannabinoid CB_1

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receptor antagonist SR 141716A (Gessa et al., 1997). We now report that the cannabinoids Δ^9-tetrahydrocannabinol and WIN 55,212-2 reduce acetylcholine release not only in the hippocampus but also in the medial-prefrontal cortex and that SR 141716A not only reverses this effect but itself increases acetylcholine release, suggesting that endogenous cannabinoids exert a tonic inhibitory control on cholinergic activity both in the medial-prefrontal cortex and hippocampus.

2. Materials and methods

2.1. Surgery and microdialysis

Male Sprague–Dawley rats (200–250 g; Charles River, Como, Italy) were housed individually in plexiglass chambers ($25 \times 40 \times 15$ cm) at 22 ± 1°C and 55% 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 h.

Implantation of the microdialysis probes was performed under general anaesthesia (Equitesin 4 ml/kg i.p.) as previously reported (Imperato et al., 1992).

Briefly, two separate groups of rats were implanted with a transversal dialysis probe (AN 69-HF, tube outer diameter 320 μm; Hospal-Dasco, Bologna, Italy), passing bilaterally through the medial–prefrontal cortex (A + 2.7 and V − 3.0) and the hippocampus (A − 3.2 and V − 3.6) respectively; 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 surface length of 0.5 cm and 1 cm for the bilateral medial–prefrontal cortex and hippocampus, respectively.

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

Microdialysis perfusions were performed 24 h after implantation during the light phase. 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 buffer 1.5 pH 7.3. Neostigmine at the final concentration of 0.1 mM was added in order to recover detectable concentrations of dialysate acetylcholine. Samples (40 μl), collected every 20 min, were analysed by high performance liquid chromatography with electrochemical detection (Damsma and Westerink, 1991).

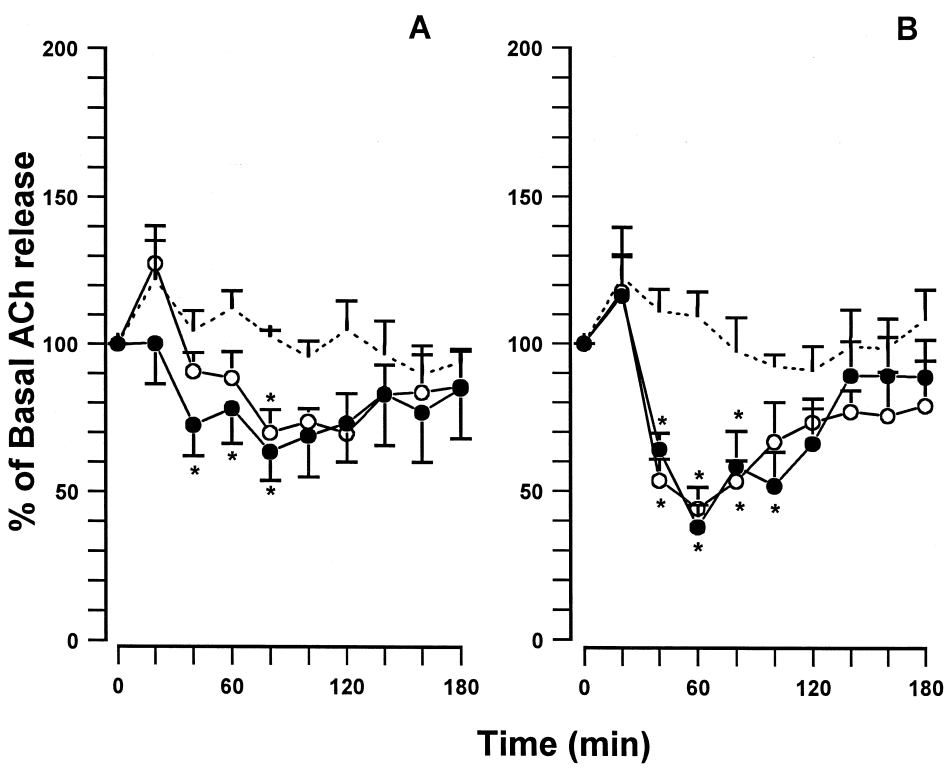

Fig. 1. Effect of Δ^9-tetrahydrocannabinol at doses of 1 (○) and 5 (●) mg/kg i.p., (panel A) and WIN 55,212-2 at doses of 5 (○) and 10 (●) mg/kg i.p., (panel B) on acetylcholine release in the medial–prefrontal cortex. Data are expressed as mean (±S.E.M.) percent variations of basal values from 4–12 rats. $*$ $P < 0.05$ vs. vehicle (Dunnett's test). For the effect of Δ^9-tetrahydrocannabinol, ANOVA revealed a significant main effect of treatment ($F(2,12) = 3.755$; $P < 0.05$); a significant time effect [$F(8,96) = 4.75$; $P < 0.0001$]; no significant time × groups interaction was observed $F(8,16) = 0.672$. For the effect of WIN 55,212-2, ANOVA revealed a significant main effect of treatment ($F(2,11) = 5.469$; $P < 0.02$); a significant time effect ($F(8,88) = 9.838$; $P < 0.0001$) and a significant time × groups interaction was observed $F(8,16) = 1.800$ $P < 0.04$.

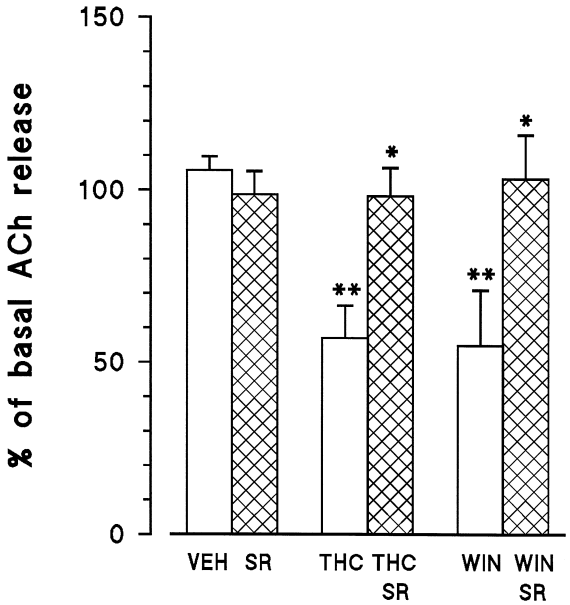

Fig. 2. Effect of SR 141716A (0.1 mg/kg i.p) on the reduction in medial–prefrontal cortex acetylcholine release induced by Δ^9-tetrahydrocannabinol (5 mg/kg i.p.) and by WIN 55,212-2 (10 mg/kg i.p.) given i. p. 5 min. before cannabinoid agonist; data were obtained from dialysates 60 min. after treatment. Data are expressed as mean (±S.E.M.) percent variation of basal values from 4–12 rats. $* P < 0.05$ vs. corresponding pre-treated group; $** P < 0.01$ vs. vehicle (Newman–Keuls test). ANOVA revealed a significant main effect of treatment ($F(5,32) = 6.1$; $P < 0.01$).

2.2. Drugs and treatments

Δ^9-tetrahydrocannabinol (RBI, Italy) was prepared from vials containing 10 mg in 1 ml of absolute ethanol. This solution was evaporated under nitrogen and the residue dissolved in two drops of Tween 80 and diluted in saline solution.

SR 141716A {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3carboxamide} HCl, (Sanofi Recherche, Montpellier, France) was dissolved in two drops of Tween 80 and diluted in saline solution.

WIN 55,212-2 {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinylmethyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-y)(1-naphthalenyl)methanone monomethanesulfonate} (RBI, Italy) was dissolved in 10% Cremophor El (Sigma, Italy), and diluted in saline solution.

Drugs were administered intrapenitoneally (i.p.) in a volume of 3 ml/kg.

Control rats were treated with the vehicle used to dissolve the active ingredient.

2.3. Statistical analysis

The average concentration of acetylcholine in the last three samples before treatment was taken as 100% and all subsequent post-treatment values were expressed as means ± S.E.M. of percentage of variations from basal values.

Between-group comparisons were performed by One-way analysis of variance for repeated measures (ANOVA). Post-hoc comparisons were performed by Newman–Keuls and by Dunnett's tests.

3. Results

3.1. Medial–prefrontal cortex

The average baseline extracellular acetylcholine concentrations (±S.E.M.) were 2.2 ± 0.10 pmol/sample. No significant differences in the baseline output between experimental groups were observed. As shown in Fig. 1A, the administration of Δ^9-tetrahydrocannabinol at the doses of 1 and 5 mg/kg produced a dose-related inhibition of acetylcholine release. Maximum reduction (about 40%) was observed at 80 min and the reduction persisted up to 120 min after treatment. A lower dose of Δ^9-tetrahydrocannabinol (0.5 mg/kg i.p.) did not significantly modify acetylcholine release (data not shown).

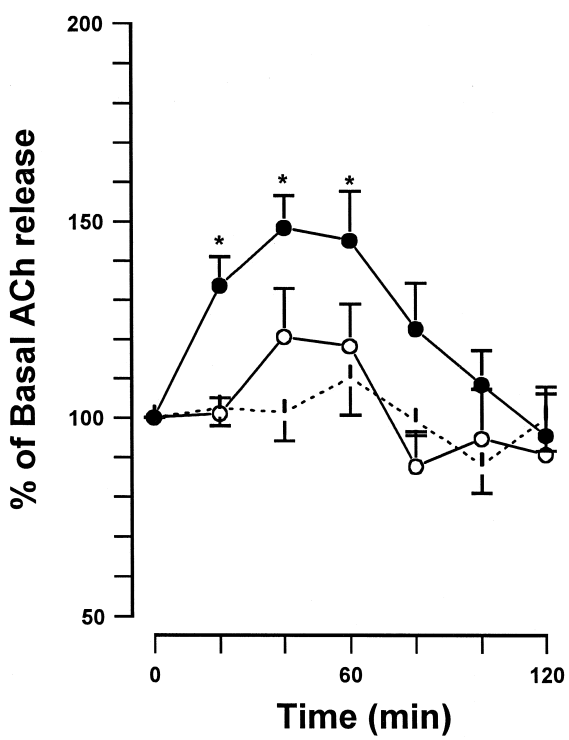

Fig. 3. Effect of SR 141716A 1.0 (○) and 3.0 (●) mg/kg i.p. on acetylcholine release in the medial–prefrontal cortex. Data are expressed as mean (±S.E.M.) percent variation of basal values from 4–12 rats. $* P < 0.05$ vs. vehicle (Dunnett's test). ANOVA revealed a significant main effect of treatment ($F(2,12) = 5.205$; $P < 0.02$); a significant time effect ($F(5,60) = 7.224$; $P < 0.0001$); no significant time × groups interaction was observed $F(5,10) = 1.393$.

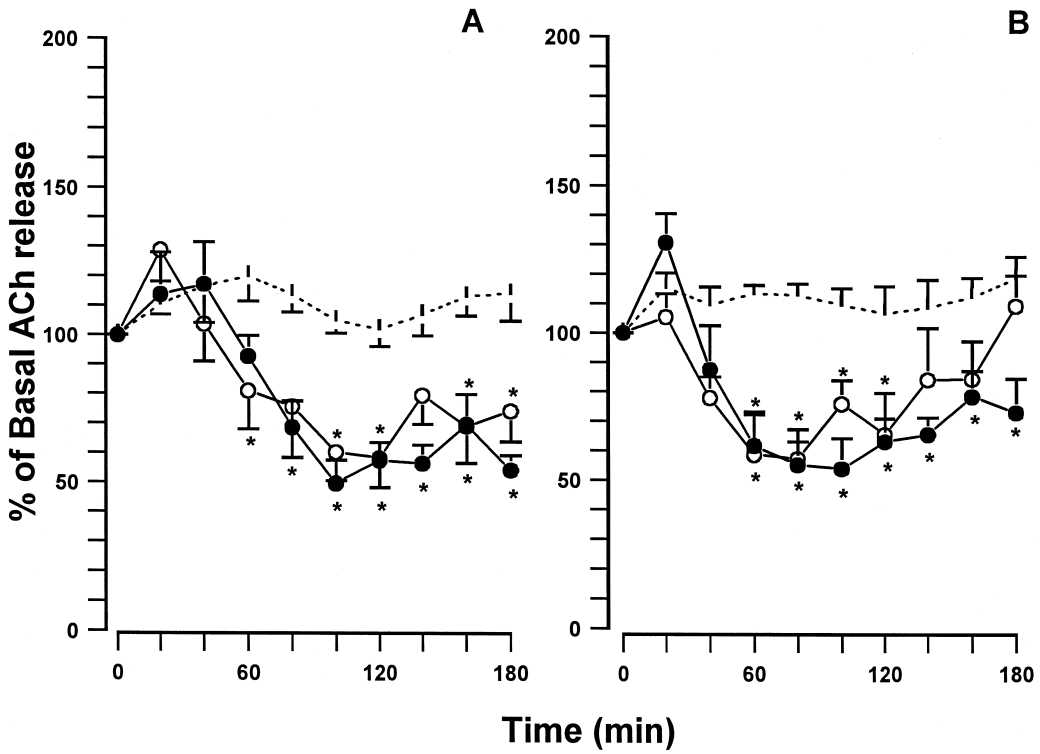

Fig. 4. Effect of Δ^9-tetrahydrocannabinol 1 (○) and 5 (●) mg/kg i.p. (panel A) and WIN 55,212-2 5 (○) and 10 (●) mg/kg i.p. (panel B), on acetylcholine release in the hippocampus. Data are expressed as mean (±S.E.M.) percent variation of basal values from 4–12 rats. $*$ $P < 0.05$ vs. vehicle (Dunnett's test). For the effect of Δ^9-tetrahydrocannabinol, ANOVA revealed a significant main effect of treatment ($F(2,11) = 6.965$; $P < 0,01$); a significant time effect ($F(8,88) = 12.867$; $P < 0.0001$) and a significant time × groups interaction ($F(8,16) = 2.937$; $P < 0.001$). For the effect of WIN 55,212-2 ANOVA revealed a significant main effect of treatment ($F(5,11) = 4.392$; $P < 0.019$); a significant time effect ($F(8,88) = 11.571$; $P < 0.0001$) and a significant time × groups interaction ($F(8,40) = 3.666$ $P < 0.0001$).

Similarly, the administration of WIN 55,212-2 at the doses of 5.0 and 10 mg/kg produced a maximal inhibition of acetylcholine release of about 60% within 60 min and the inhibition lasted for about 2 h (Fig. 1B).

The effect of Δ^9-tetrahydrocannabinol and WIN 55,212-2 was prevented by the specific cannabinoid receptor antagonist, SR 141716A, at the dose of 0.1 mg/kg, ineffective per se to modify acetylcholine release (Fig. 2). Most interestingly, SR 141716A alone, at the dose 3 mg/kg, increased acetylcholine release by about 45% within 20 min after treatment and the increase persisted up to 60 min. after treatment. Lower dose of SR 141716A (1.0 mg/kg i.p.) failed to modify acetylcholine release (Fig. 3).

3.2. Hippocampus

The average baseline extracellular acetylcholine concentrations (±S.E.M.) were 1.68 ± 0.17 pmol/sample. No significant differences in the baseline output between experimental groups were observed. The administration of Δ^9-tetrahydrocannabinol (1.0 and 5.0 mg/kg i.p.) reduced acetylcholine release by about 50% within 80 min; the inhibition persisted over the entire observation period (Fig. 4A).

Consistent with our previous observation (Gessa et al., 1997), the administration of WIN 55,212-2 at the dose of 5 and 10 mg/kg reduced hippocampal acetylcholine release by about 45% within 60 min. The reduction produced by the higher dose persisted over the entire observation period (Fig. 4B).

As in the medial-prefrontal cortex, the effect of both Δ^9-tetrahydrocannabinol (5 mg/kg) and WIN 55,212-2 (10 mg/kg) was prevented by SR 141716A at the dose of 0.1 mg/kg, itself ineffective to modify acetylcholine release (Fig. 5). Moreover, SR 14716A alone at the doses of 1.0 and 3.0 mg/kg, increased acetylcholine release by about 45 % within 20 min, the increase persisting for 80–120 min (Fig. 6).

4. Discussion

The present study shows that Δ^9-tetrahydrocannabinol, the psychoactive principle of marijuana, and WIN 55,212-2, a synthetic cannabinoid receptor agonist, produce a long lasting inhibition of acetylcholine release both in the medial–prefrontal cortex and hippocampus. Our results extend our previous report showing that besides WIN 55,212-2, also CP 55,940, another synthetic cannabinoid

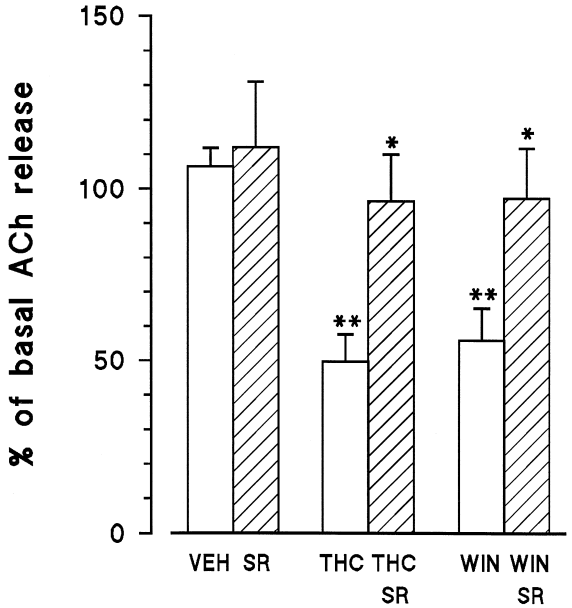

Fig. 5. Effect of SR 141716A (0.1 mg/kg i.p) on the reduction in the hippocampal acetylcholine release of induced by Δ^9-tetrahydrocannabinol (THC) (5 mg/kg) and WIN 55,212-2 (10 mg/kg) was given i.p. 5 min. before cannabinoid agonist; data were obtained from dialysates 60 min. after treatment. Data are expressed as mean (±S.E.M.) percent variation of basal values from 4–12 rats. $*\ P < 0.05$ vs. corresponding pre-treated group; $**\ P < 0.01$ vs. vehicle (Newman–Keuls test). ANOVA revealed a significant main effect of treatment ($F(5,36) = 5.35$; $P < 0.01$).

receptor agonists, inhibits acetylcholine release in the hippocampus (Gessa et al., 1997). Moreover they are in accord with studies showing that cannabinoid receptor agonists inhibit electrically-evoked [^{14}C]acetylcholine release from hippocampal and striatal slices (Gifford and Ashby, 1996, Gifford et al., 1997), and from guinea pig myenteric plexus (Coutts and Pertwee, 1997).

The inhibitory effect of WIN 55,212-2 and Δ^9-tetrahydrocannabinol on hippocampal and medial–prefrontal cortex acetylcholine release is most likely mediated through cannabinoid CB_1 receptors, as it is produced by structurally distinct cannabinoid receptor agonists and it is suppressed by the specific cannabinoid CB_1 receptor antagonist, SR 141716A. The involvement of cannabinoid CB_1 receptors in the control of acetylcholine release might be located on cholinergic nerve terminals in the hippocampus and medial–prefrontal cortex as suggested by the above mentioned experiments in striatal slices (Gifford et al., 1997) and by autoradiographic and immunohistochemical studies showing cannabinoid CB_1 receptors in the cortex and in the hippocampus (Tsou et al., 1997). Moreover, the high level of cannabinoid CB_1 receptor mRNA in the septum and diagonal band of Broca, where hippocampal cholinergic neurons originate, support the contention that cannabinoid CB_1 receptors are located on cholinergic nerve terminals (Mailleux and Vanderhaeghen, 1992). However, since high levels of cannabinoid CB_1 receptor mRNA were also found in the hippocampus (Gifford et al., 1997), it is possible that intrinsic neurons might play a role in the control of cholinergic transmission by cannabinoid.

By perfusing the hippocampus and medial–prefrontal cortex with cannabinoids via the dialysis probe it may clarify if these areas are the target of cannabinoid action.

An important outcome of our study is that SR 141716A itself increases acetylcholine release both in the hippocampus and in the medial–prefrontal cortex. This finding is in agreement with previous observations that SR 141716A alone, enhanced electrically evoked acetylcholine release in the rat hippocampus (Gifford and Ashby, 1996) and in the guinea pig myenteric plexus (Coutts and Pertwee, 1997).

Two explanations may be suggested to account for the SR 141716A effect. This compound may be either antagonising the action of endogenous cannabinoids that tonically inhibit acetylcholine release, or the stimulant effect of SR 141716A might depend on an inverse agonist activity of the compound (Rinaldi-Carmona et al., 1994). The latter possibility would imply that cannabinoid CB_1 receptors controlling acetylcholine release are constitutively active in inhibiting such release.

In addition to inhibiting acetylcholine release, cannabinoid agonists activate the firing rate of mesolimbic and mesocortical dopaminergic neurons (Gessa et al., 1997;

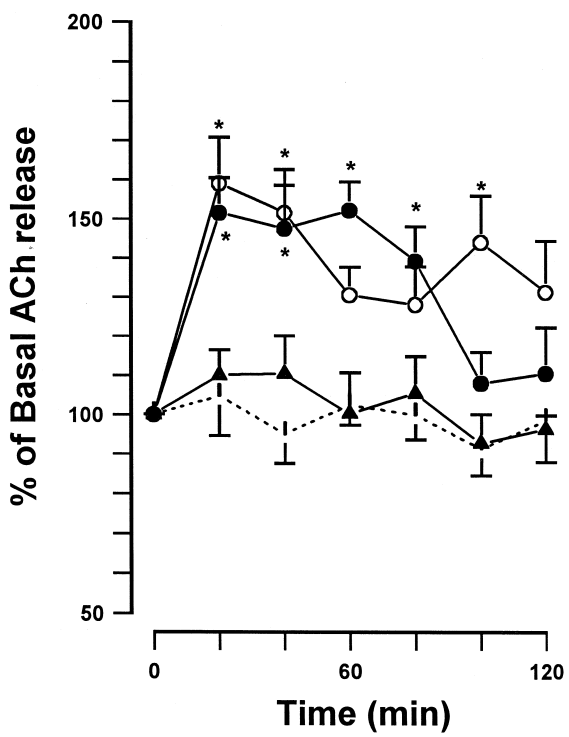

Fig. 6. Effect of SR 141716A 0.1 (♦), 1.0 (○) and 3.0 (●) mg/kg i.p. on acetylcholine release in the hippocampus. Data are expressed as mean (±S.E.M.) percent variation of basal values from 4–12 rats. $*\ P < 0.05$ vs. vehicle (Dunnett's test). ANOVA revealed a significant main effect of treatment ($F(3,17) = 4.780$; $P < 0.014$); a significant time effect ($F(5,85) = 6.016$; $P < 0.0001$) and a significant time × groups interaction ($F(5,15) = 2.276$ $P < 0.009$).

Diana et al., 1998). This effect would be expected to increase, instead of decreasing, acetylcholine release in the hippocampus (Imperato et al., 1993a,b) and medial–prefrontal cortex (Day and Fibiger, 1992).

To explain this apparent discrepancy one might suggest that the direct inhibitory effect of cannabinoids on cholinergic nerve terminals might override the stimulant effect of dopamine.

Since acetylcholine is considered to be involved not only in learning and memory processes but, more specifically, in attentional processes (Blokland, 1996), the problem arises as to whether the inhibitory effect of cannabinoid receptor agonists on acetylcholine release in the hippocampus and medial–prefrontal cortex might represent the neurochemical substrate for the cognitive impairments caused by this compounds. Against this possibility, Lichtman and Martin found that the cholinesterase inhibitor, physostigmine, unlike SR 141716A fails to improve Δ^9-tetrahydrocannabinol induced deficits in working memory (Lichtman and Martin, 1996).

Therefore, future research is needed to establish if the inhibition of cholinergic transmission is involved in cannabinoid effect. Clarifying this issue is clinically relevant since the inhibitory effects of Δ^9-tetrahydrocannabinol on acetylcholine release are observed with doses within those found in humans use of marijuana (Davis et al., 1984), and vice versa, cannabinoid CB_1 receptor antagonists might offer potential treatments for cognitive deficits (Terranova et al., 1996).

Acknowledgements

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