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Δ^9-tetrahydrocannabinol enhances cortical and hippocampal acetylcholine release in vivo: a microdialysis study

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Abstract

The intravenous administration of synthetic cannabinoid agonists was recently shown to dose dependently increase acetylcholine release from the rat prefrontal cortex and hippocampus (Eur. J. Pharmacol. 401 (2000) 179]. We report here that the active ingredient of cannabis preparations, Δ^9-tetrahydrocannabinol, administered at 10, 37.5, 75 and 150 μg/kg, dose dependently stimulated acetylcholine release from rat prefrontal cortex and hippocampus estimated by means of in vivo brain microdialysis with vertical concentric probes. At these doses, Δ^9-tetrahydrocannabinol induced behavioural stimulation. The administration of the CB_1 receptor antagonist, ({*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3carboxamide}HCl) SR 141716A (200 μg/kg i.p.) significantly reduced the effect of Δ^9-tetrahydrocannabinol (75 μg/kg i.v.) on acetylcholine release from rat prefrontal cortex and hippocampus.

Keywords: Acetylcholine; Hippocampus; Δ^9-tetrahydrocannabinol; Microdialysis, in vivo; Cortex, prefrontal; SR 141716A

1. Introduction

Cortical and hippocampal acetylcholine neurotransmission is stimulated in relation to arousal (Marrosu et al., 1995; Metherate et al., 1992) and focused attention (Acquas et al., 1996; Passetti et al., 2000; Voytko, 1996).

Δ^9-Tetrahydrocannabinol, anandamide (the putative endogenous ligand for cannabinoid receptors) and synthetic cannabinoid receptor agonists show biphasic effects on behaviour (Sanudo-Pena et al., 2000; Sulcova et al., 1998) and on brain metabolism (Margulies and Hammer, 1991). On the basis of in vitro (Gifford and Ashby, 1996) and in vivo studies (Carta et al., 1998; Gessa et al., 1998), it has been concluded that cannabinoids control acetylcholine release in an inhibitory manner via cannabinoid CB_1 receptors. However, low intravenous doses of the synthetic cannabinoid receptor agonists, ((*R*(+)-[2,3-Dihydro-5-methyl-3-[morpholinyl)methyl] pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate)) WIN 55,212-2 and (((6*aR*)-*trans*-3-(1,1-Dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol)) HU 210 have been reported to enhance acetylcholine release from the rat prefrontal cortex and hippocampus (Acquas et al., 2000) at doses that have been shown to preferentially enhance dopamine release in the nucleus accumbens shell (Tanda et al., 1997).

The aim of the present study was to further explore the role of cannabinoid CB_1 receptors in the modulation of acetylcholine release from the frontal cortex and hippocampus through the intravenous administration of low doses of Δ^9-tetrahydrocannabinol.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (275–300 g) were housed in groups of two to three per cage for at least 3 days before use and were maintained on a 12:00/12:00-h light/dark cycle (lights on at 7:30 AM) with food and water available ad libitum. After surgery, the rats were individually housed in hemispherical bowls which also served as the experimental environment. Experiments were carried out be-

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tween 9:00 AM and 4:00 PM at least 24–30 h after the surgery.

2.2. Surgery and microdialysis

Rats were anaesthetized with ketamine HCl (Ketalar, Parke Davis, Italy) (100 mg/kg i.p.) and stereotaxically implanted with concentric microdialysis probes aimed at the prefrontal cortex (AP = +3.6 mm, DV = −4.8 mm, ML = −0.7) and at the hippocampus (AP = −5.5 mm, DV = −5.5 mm, ML = +5.0) according to Paxinos and Watson (1986). For intravenous administration, under halotane anaesthesia rats were implanted, in the same day with a polyethylene catheter in the left femoral vein and tunneled subcutaneously to exit at the nape of the neck according to Crane and Porrino (1989). The membrane for microdialysis, a polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospal, Italy), was covered with epoxy glue along its whole length except for 3 mm corresponding to the area of dialysis. The day of the experiment rats were connected to a 2.5-ml glass syringe containing normal Ringer (147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$, with the addition of the reversible acetylcholine esterase inhibitor, neostigmine bromide (0.1 μM) (Sigma, MO, USA) in twice distilled water. Perfusion flow was set at 1.25 μl/min. Samples were collected every 10 min into a 20-μl sample loop and subsequently injected in a high-pressure liquid chromatography (HPLC) injector valve. Acetylcholine was assayed by HPLC coupled with electrochemical detection in conjunction with an enzyme reactor (Damsma et al., 1987). Acetylcholine was separated on a reverse phase Chromspher C_{18} 5 μm (Merck, FRG) column (75 × 2.1 mm). The mobile phase passed directly through the enzyme reactor containing acetylcholine esterase (ED 3.1.1.7; type VI-S, Sigma, MO, USA) and choline oxidase (EC 1.1.3.17; Sigma). Acetylcholine was quantitatively converted into hydrogen peroxide, which was detected electrochemically at a platinum working electrode set at 500 mV versus an Ag/AgCl reference electrode (LC-4B, BAS, IN, USA). The mobile phase (1.9 mM K_2HPO_4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) was delivered constantly at 0.4 ml/min by an HPLC pump. The detection limit of the assay was about 50 fmol/sample. Injections of an acetylcholine standard (20 μl, 0.1 μM) were made every 60 to 90 min in order to monitor changes in electrode sensitivity and sample concentrations were corrected accordingly.

2.3. Behavioural measures

Spontaneous behaviour was classified as: *Still, Sedated:* resting, lying down with eyes closed or half open; *Still, Aroused:* eyes wide open, movements of the head and of the whiskers, chewing; *Active:* upward sniffing and rearing, locomotor activity accompanied by sniffing (exploratory behaviour), wet-dog shakes, digging in the bedding, grooming.

2.4. Drugs

Δ^9-Tetrahydrocannabinol (NIDA, Baltimore, MD, USA) and {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide}HCl, SR 141716A (Sanofi Research, France), were suspended in 3% TWEEN 80 in saline and administered intravenously (Δ^9-tetrahydrocannabinol) or intraperitoneally (SR 141716A) in a volume of 1 ml/kg.

2.5. Statistics

Values are expressed as percent changes with respect to baseline (100%). Baseline was set as the average of the last six pre-treatment samples, not differing more than 15%. One-way, two-way and three-way analyses of variance (ANOVA), with time as the repeated measure, were used to analyse the treatment effects. Tukey's post hoc analyses were applied for multiple comparisons, with the statistical significance set at $P < 0.05$.

3. Results

3.1. Basal acetylcholine output and effect of Δ^9-tetrahydrocannabinol on prefrontal cortical and hippocampal acetylcholine release

The overall mean ± S.E.M. baseline levels of acetylcholine in the dialysates from the prefrontal cortex and hippocampus was 69 ± 2 fmol/min ($n = 64$) and 35 ± 5 fmol/min ($n = 61$), respectively. As shown in Fig. 1, intravenous administration of vehicle did not affect basal acetylcholine output (one-way ANOVA, prefrontal cortex (top left panel): $F(9,72) = 1.12$, NS; hippocampus (bottom left panel): $F(9,72) = 1.28$, NS). Δ^9-Tetrahydrocannabinol stimulated acetylcholine output in the prefrontal cortex at the dose of 37.5 μg/kg ($F(4,16) = 3.14, P < 0.04$) and in the prefrontal cortex and in the hippocampus at the dose of 75 μg/kg (prefrontal cortex: ($F(9,36) = 4.48$, $P < 0.001$); hippocampus: ($F(9,45) = 3.17$, $P < 0.0047$), and at the dose of 150 μg/kg (prefrontal cortex: $F(9,54) = 4.16$, $P < 0.001$; hippocampus: $F(9,81) = 5.8$, $P < 0.001$), while changes in acetylcholine output did not reach statistical significance at the dose of 10 μg/kg in the prefrontal cortex, $F(9,54) = 0.7$, NS and in the hippocampus ($F(9,63) = 1.12$, NS) and at the dose of 37.5 μg/kg in the hippocampus ($F(9,45) = 0.86$, NS). Right panels of Fig. 1 show the cumulative % changes of acetylcholine output in the first four samples after intravenous administration of vehicle or Δ^9-tetrahydrocannabinol (10, 37.5, 75 and 150

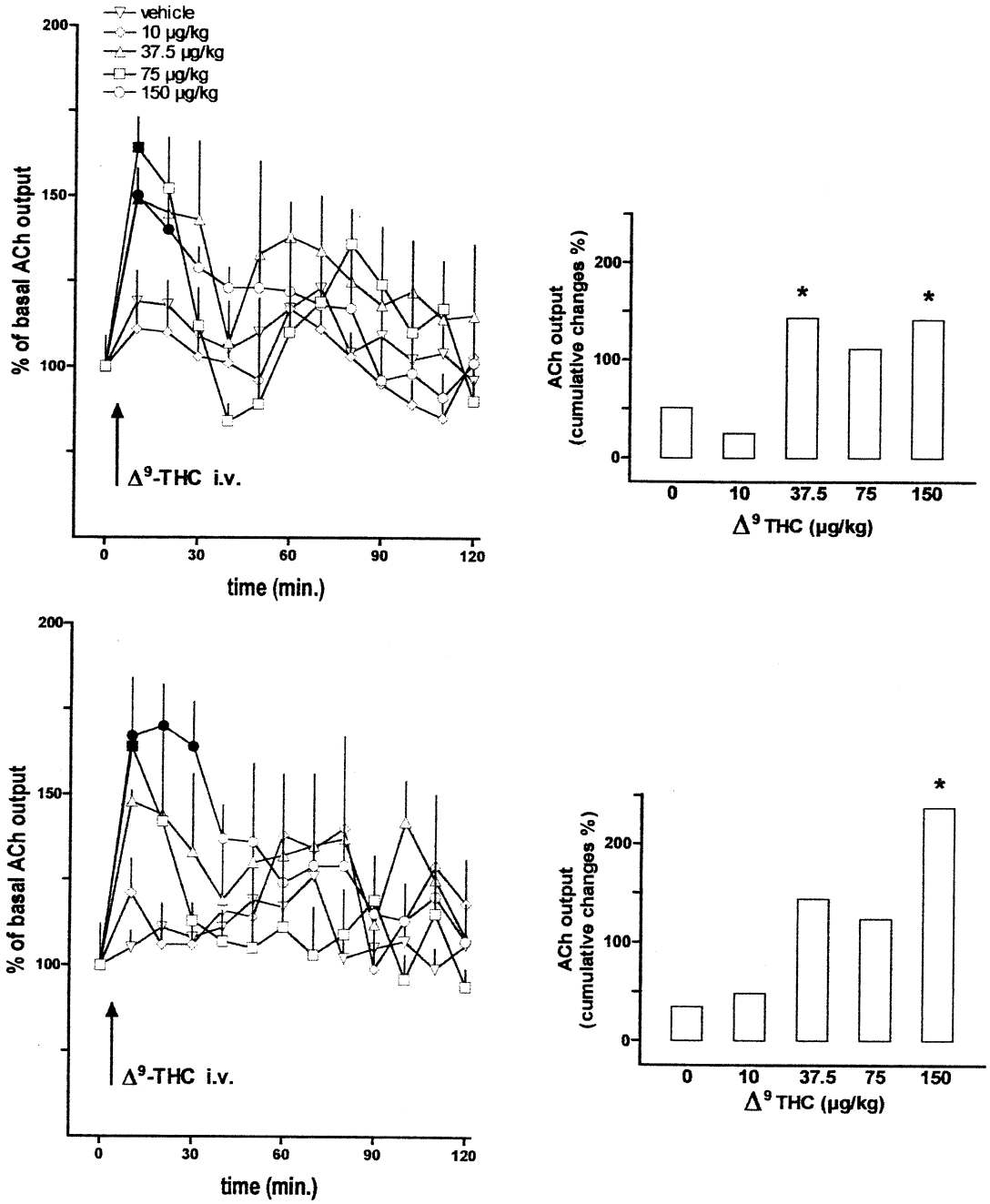

Fig. 1. Left panels (top): effects of vehicle ($n = 9$) and of Δ^9-tetrahydrocannabinol (Δ^9-THC) (10, 37.5, 75 and 150 μg/kg i.v.) ($n = 7$, 6, 5 and 7, respectively) on prefrontal cortical acetylcholine release; (bottom): effects of vehicle ($n = 9$) and of Δ^9 tetrahydrocannabinol (Δ^9-THC) (10, 37.5, 75 and 150 μg/kg i.v.) ($n = 8$, 6, 6, and 10, respectively) on hippocampal acetylcholine release. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. Filled symbols indicate samples significantly different from baseline ($P < 0.05$ at Tukey's post hoc test). Right panels: histograms represent the cumulative % changes of acetylcholine output during the first four samples after vehicle or Δ^9-tetrahydrocannabinol (Δ^9-THC) administration. * $P < 0.05$ versus the vehicle + vehicle group.

μg/kg i.v.). The increase of acetylcholine output by Δ^9-tetrahydrocannabinol was dose-dependent as shown by significant main effect of dose (prefrontal cortex: $F(4,28) = 4.22$, $P < 0.008$; hippocampus $F(4,34) = 3.34$, $P < 0.02$) and significance of Tukey's post hoc test ($P < 0.05$) for differences between vehicle + vehicle versus vehicle + Δ^9-tetrahydrocannabinol (37.5 or 150 μg/kg i.v.) in the prefrontal cortex and between vehicle + vehicle versus vehicle + Δ^9-tetrahydrocannabinol (150 μg/kg i.v.) in the hippocampus.

3.2. Effect of SR 141716A on basal and Δ^9-tetrahydrocannabinol-evoked acetylcholine output from prefrontal cortex and hippocampus

Fig. 2 shows the effects of vehicle or SR 141716A (200 μg/kg) administered i.p. 30 min beforehand on the in-

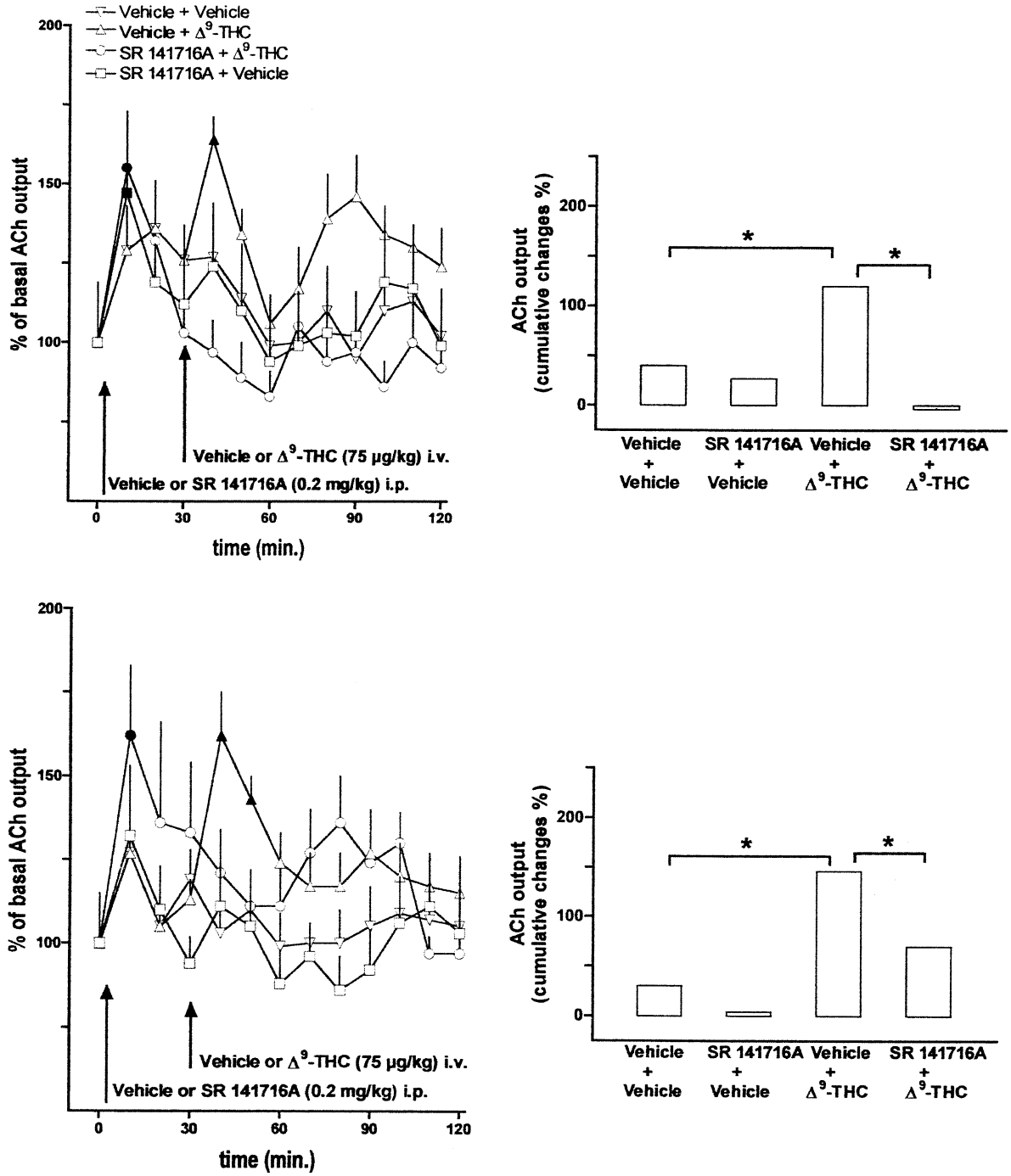

Fig. 2. Left panels (top): effects of vehicle (1 ml/kg) or SR 141716A (200 μg/kg i.p.), followed 30 min later by intravenous administration of vehicle (1 ml/kg) ($n = 7$ and 4, respectively) or Δ^9-tetrahydrocannabinol (Δ^9-THC) (75 μg/kg) ($n = 5$ and 14) on basal prefrontal cortical acetylcholine release; (bottom): effects of vehicle (1 ml/kg i.p.) or SR 141716A (200 μg/kg i.p.), followed 30 min later by intravenous administration of vehicle (1 ml/kg i.p.) ($n = 6$ and 4, respectively) or Δ^9-tetrahydrocannabinol (Δ^9-THC) (75 μg/kg) ($n = 4$ and 8) on basal hippocampal acetylcholine release. Values are expressed as percentage baseline. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. Filled symbols indicate samples significantly different from baseline ($P < 0.05$ at Tukey's post hoc test). Right panels: histograms represent the cumulative % changes of acetylcholine output during the first four samples after vehicle or Δ^9-tetrahydrocannabinol (Δ^9-THC) administration. $^*P < 0.05$ versus the vehicle + vehicle group.

crease of cortical (upper panels) and hippocampal (lower panels) acetylcholine release evoked by Δ^9-tetrahydrocannabinol (75 μg/kg i.v.). As shown in top and bottom left panels, the intraperitoneal administration of vehicle increased acetylcholine release from the prefrontal cortex ($F(3,18) = 5.2$, $P < 0.008$) but not from the hippocampus ($F(3,6) = 1.63$, NS), while the intraperitoneal administration of SR 141716A increased basal acetylcholine output

in the prefrontal cortex and in the hippocampus (prefrontal cortex: $F(9,36) = 6.33$, $P < 0.00001$; hippocampus: $F(9,27) = 2.26$, $P < 0.048$). Two-way ANOVA, however, did not show a significant effect of treatment for the first three samples after i.p. administration of SR 141716A or vehicle (prefrontal cortex: $F(1,11) = 4.1$, NS; hippocampus: $F(1,11) = 3.4$, NS). The administration of Δ^9-tetrahydrocannabinol (75 μg/kg), following vehicle administration, increased basal acetylcholine output in the prefrontal cortex ($F(6,66) = 3.76$, $P < 0.002$) and in the hippocampus ($F(4,44) = 3.5$, $P < 0.014$). Two-way ANOVA provided a significant main effect of Δ^9-tetrahydrocannabinol in the groups vehicle + Δ^9-tetrahydrocannabinol and vehicle + vehicle (prefrontal cortex: $F(1,12) = 6.61$; $P < 0.02$; hippocampus: $F(1,10) = 24.9$; $P < 0.0005$). In animals pretreated with SR 141716A (200 μg/kg i.p.), Δ^9-tetrahydrocannabinol (75 μg/kg) failed to modify acetylcholine output in the prefrontal cortex and in the hippocampus (prefrontal cortex: $F(6,24) = 2.49$, NS; hippocampus: $F(4,12) = 2.62$, NS); two-way ANOVA revealed a significant main effect of SR 141716A on acetylcholine release stimulated by Δ^9-tetrahydrocannabinol in the prefrontal cortex ($F(1,15) = 20.64$, $P < 0.05$) and in the hippocampus ($F(1,14) = 6.46$, $P < 0.023$). Top and bottom right panels of Fig. 2 show the cumulative % changes of acetylcholine output above baseline during the first four samples after intravenous administration of vehicle or Δ^9-tetrahydrocannabinol from SR 141716A or vehicle pretreated rats. Three-way ANOVA with pretreatment and treatment as dependent variables and time as independent variable yielded a significant pretreatment × treatment × time interaction in the prefrontal cortex ($F(3,57) = 3.22$; $P < 0.029$) and in the hippocampus ($F(3,51) = 4.47$, $P < 0.007$); Tukey's post hoc test showed that Δ^9-tetrahydrocannabinol increased acetylcholine in dialysates to a lesser extent after pretreatment with SR 141716A.

3.3. Behavioural effects of Δ^9-tetrahydrocannabinol, SR 141716A and SR 141716A + Δ^9-tetrahydrocannabinol

Fig. 3 shows the behavioural effects of Δ^9-tetrahydrocannabinol administration. Δ^9-tetrahydrocannabinol (10 μg/kg i.v.) elicited a short-lasting (< 10 min) behavioural activation characterized by chewing, fine movements of the head and of the whiskers (*Still, aroused*) and by short episodes of upward sniffing and grooming. The administration of Δ^9-tetrahydrocannabinol at the dose of 37.5 μg/kg i.v. elicited behavioural activation characterized by upward sniffing and rearing, followed by grooming, which lasted for 10 to 20 min At the higher doses (75 and 150 μg/kg i.v.), Δ^9-tetrahydrocannabinol elicited a more consistent pattern of behavioural activation with digging in the bedding, wet-dog shakes, locomotion and upward sniffing (exploratory behaviour) for 20 to 30 min after Δ^9-tetrahydrocannabinol administration, followed by grooming. Intraperitoneal administration of SR 141716A or vehicle elicited short-lasting locomotion within the first 10 min after injection. On the other hand, SR 141716A (200 μg/kg i.p.) prevented the behavioural activation induced by Δ^9-tetrahydrocannabinol (75 μg/kg i.v.).

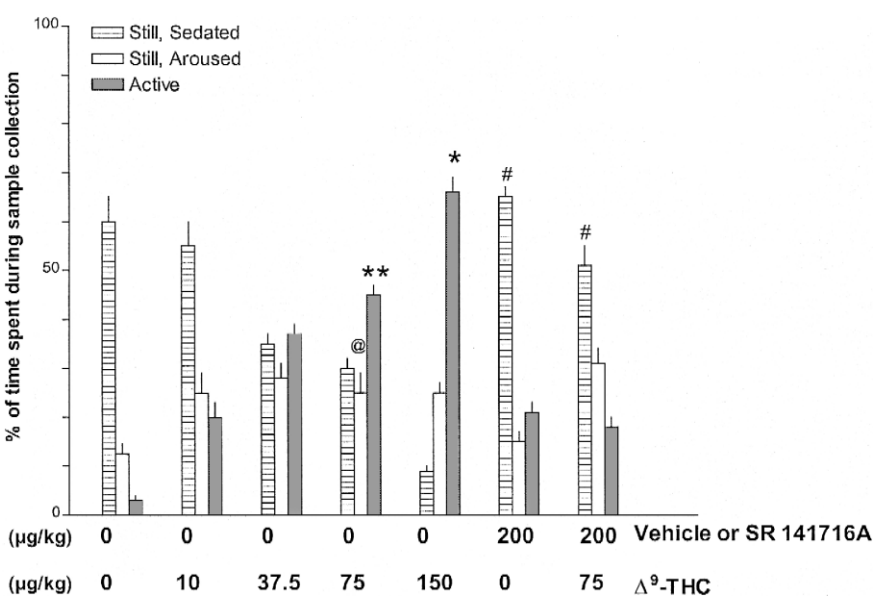

Fig. 3. Behavioural effects of vehicle, Δ^9-tetrahydrocannabinol (Δ^9-THC) and of Δ^9-THC on vehicle or SR 141716A-pretreated rats. See behavioural items in Section 3. Histograms represent the % of the time spent during the collection of the first four samples after vehicle or Δ^9-THC administration. Data obtained from rats of the vehicle and vehicle + vehicle groups and from the Δ^9-THC (75 μg/kg) and the vehicle + Δ^9-THC (75 μg/kg) groups were pooled. Still, Sedated: $F_{(1.6)} = 28$; $P < 0.0001$, $^{\#}P < 0.01$ *versus* vehicle + Δ^9-THC (75 or 150 μg/kg) groups (Tukeys' test); Still, Aroused: $F_{(1.6)} = 55$; $P < 0.0002$, $^{@}P < 0.05$ *versus* vehicle + vehicle and SR 141716A + vehicle groups (Tukeys' test); Active: $F_{(1.6)} = 114$; $P < 0.0001$; $^{*}P < 0.05$ *versus* vehicle or SR 141716A + Δ^9-THC (75 μg/kg); $^{**}P < 0.05$ *versus* SR 141716A + Δ^9-THC (75 μg/kg) groups (Tukeys' test).

4. Discussion

Δ^9-tetrahydrocannabinol dose dependently enhanced cortical and hippocampal acetylcholine release after intravenous doses of 75 and 150 μg/kg. This effect was mediated by cannabinoid CB_1 receptors since pretreatment with SR 141716A prevented it. The findings of the present study are in line with our recent report that low, intravenous doses of the synthetic cannabinoid receptor agonists WIN 55,212-2 and HU 210 increase cortical and hippocampal acetylcholine release (Acquas et al., 2000).

On the basis of in vitro studies on electrically evoked acetylcholine release from hippocampal slices (Gifford and Ashby, 1996) and of in vivo microdialysis studies of acetylcholine release from rat frontal cortex and hippocampus (Carta et al., 1998; Gessa et al., 1998), it has been concluded that Δ^9-tetrahydrocannabinol and synthetic cannabinoid receptor agonists decrease acetylcholine output and it was suggested that such decreases could be responsible for the memory and cognitive impairment induced by Δ^9-tetrahydrocannabinol (Carta et al., 1998; Gessa et al., 1998). The present findings, together with those of a previous study (Acquas et al., 2000) do not support the above conclusions. The reason for the discrepancies between our findings (this study and Acquas et al., 2000 and those of Carta et al., 1998), and Gessa et al., 1998, should be searched in experimental differences such as route of administration and doses of Δ^9-tetrahydrocannabinol employed. In relation to this, it might be worth considering that Δ^9-tetrahydrocannabinol and synthetic cannabinoid receptor agonists are known to exert biphasic effects on behaviour and brain metabolism; thus, while low doses of Δ^9-tetrahydrocannabinol and synthetic cannabinoid receptor agonists elicit stimulatory effects on behaviour (Acquas et al., 2000; Sanudo-Pena et al., 2000) and 2-deoxy-D-glucose uptake (Margulies and Hammer, 1991), high doses of Δ^9-tetrahydrocannabinol decrease 2-deoxy-D-glucose uptake (Margulies and Hammer, 1991) and reduce spontaneous motor activity (Sanudo-Pena et al., 2000). Δ^9-Tetrahydrocannabinol, in a range of doses superimposable to those utilized here, stimulates dopamine release in the shell of the nucleus accumbens (Tanda et al., 1997). This action of Δ^9-tetrahydrocannabinol might indirectly stimulate acetylcholine release as a result of its arousing and behavioural activating effects. Higher doses of Δ^9-tetrahydrocannabinol, administered i.p., might exert opposite effects and reduce acetylcholine release, as reported by previous studies.

Cannabinoids, given at doses higher than those utilised here impair short-term (Mallet and Beninger, 1996; Nakamura et al., 1991; Presburger and Robinson, 1999) but not long-term memory in rats (Mallet and Beninger, 1996; Nakamura et al., 1991). However, Δ^9-tetrahydrocannabinol is self-administered at doses even lower than those utilised here, in the range of 1–4 μg/kg i.v. by squirrel monkeys (Tanda et al., 2000). Although a relationship between acetylcholine transmission and cannabinoid-induced impairment of working memory has been excluded (Lichtman and Martin, 1996; Presburger and Robinson, 1999), the relationship between the increase in acetylcholine release and cognitive effects of cannabinoids is unknown. Thus, given the biphasic nature of Δ^9-tetrahydrocannabinol on behaviour, it remains unclear which changes in working memory are associated with the changes in acetylcholine release induced by such low doses of Δ^9-tetrahydrocannabinol. However, since it is more likely that the doses of Δ^9-tetrahydrocannabinol inhaled by cannabis smokers are in the low dose range, the changes in acetylcholine release reported in the present study might reflect those elicited by Δ^9-tetrahydrocannabinol in humans smoking marijuana or hashish.

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