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Cannabinoid CB_1 receptor agonists increase rat cortical and hippocampal acetylcholine release in vivo

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Abstract

Intravenous administration of the cannabinoid CB_1 receptor agonists (R-(+)-[2,3-Dihydro-5-methyl-3[morpholinyl)methyl]-pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate), WIN 55,212-2 (10, 37.5, 75 and 150 μg/kg), and ((6aR)-*trans*-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol), HU 210 (1 and 4 μg/kg) dose-dependently increased acetylcholine release in dialysates from the prefrontal cortex and the hippocampus of freely moving rats. Administration of the cannabinoid receptor antagonist {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3carboxamide}HCl, SR 141716A, at a dose that per se did not affect basal acetylcholine release (2.5 μg/kg), prevented the increase of acetylcholine release by WIN 55,212-2 (150 μg/kg i.v.) or by HU 210 (4 μg/kg i.v.) in both areas. These data demonstrate that, at low i.v. doses, the synthetic cannabinoid CB_1 receptor agonists, WIN 55,212-2 and HU 210 stimulate cortical and hippocampal acetylcholine release.

Keywords: Acetylcholine; Hippocampus; HU 210; Microdialysis; Cortex, prefrontal; SR 141716A; WIN 55, 212-2

1. Introduction

Cholinergic projections of the basal forebrain/nucleus basalis magnocellularis and of the medial septum (basal forebrain cholinergic complex) to the cerebral cortex and hippocampus have long been regarded as critical for memory (Bartus et al., 1985). Current views attribute to central acetylcholine a critical role in arousal and attentional processes (Fibiger, 1991; Robbins and Everitt, 1994; Bloklland, 1999). In fact, acetylcholine release in the rat frontal cortex and hippocampus is increased by presentation of both unconditioned and conditioned sensory stimuli (Inglis and Fibiger, 1995; Acquas et al., 1996, 1998) and it has been suggested that increased acetylcholine neurotransmission in such terminal areas strengthens the salience of motivationally relevant stimuli, thus, facilitating learning and memory (Robbins and Everitt, 1994; Sarter and Bruno, 1997).

Δ^9-tetrahydrocannabinol and synthetic agonists of cannabinoid CB_1 receptors have been reported to reduce acetylcholine release in vivo from rat hippocampus (Carta et al., 1998) and frontal cortex (Carta et al., 1998; Gessa et al., 1998a) at doses known to inhibit spontaneous locomotor activity. Furthermore, in rats as well as in humans, Δ^9-tetrahydrocannabinol's amnesic effects are well-described (Lichtman and Martin, 1996; Solowij et al., 1991) and it has recently been suggested that these effects of cannabinoids might be related to reductions of acetylcholine neurotransmission (Gessa et al., 1998a). However, cannabinoid receptor agonists may disrupt performance in a working memory task also through an acetylcholine-independent mechanism (Lichtman and Martin, 1996).

Intravenous administration of low doses of Δ^9-tetrahydrocannabinol and WIN 55,212-2 stimulates motor activity and dopamine release in the shell of the nucleus accumbens (Tanda et al., 1997) and increases the firing activity of dopamine units in the ventral tegmentum (Gessa et al., 1998b). Here, we report the effect of the cannabinoid CB_1 receptor agonists, WIN 55,212-2 and HU 210, administered i.v. in the range doses and with the vehicle which we utilized in our previous studies (Tanda et al.,

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1997) on acetylcholine release in the rat prefrontal cortex and in the hippocampus. Furthermore, the effect of the cannabinoid CB_1 receptor antagonist, SR 141716A (Rinaldi-Carmona et al., 1994), alone and in combination with WIN 55,212-2 or HU 210, was studied.

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 1200:1200 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 between 9:00 am and 4:00 pm at least 24–30 h after the surgical implants.

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 and at the hippocampus, the coordinates being AP = +3.6 mm, DV = −4.8 mm, ML = −0.7, and AP = −5.5 mm, DV = −5.5 mm, ML = +5.0, according to Paxinos and Watson (1986), respectively. For intravenous administration, under halotane anaesthesia, rats were subjected in the same day to a surgery in which a polyethylene catheter was inserted in their left femoral vein and tunneled subcutaneously to exit at the nape of the neck, according to Crane and Porrino (1988). The membrane for microdialysis, a polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospal, France), 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 perfusion pump by polyethylene tubing connected to a 2.5-ml glass syringe containing normal Ringer and with perfusion flow 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. To achieve consistently detectable amounts of acetylcholine in dialysates, the reversible acetylcholine esterase inhibitor, neostigmine bromide (0.1 μM) (Sigma), was added to normal Ringer, containing 147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$, in twice distilled water. 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) and choline oxidase (EC 1.1.3.17; Sigma) covalently bound to glutaraldehyde-activated Lichrosorb 10-NH_2; acetylcholine was quantitatively converted into hydrogen peroxide which was detected electrochemically at a platinum working electrode set at 500 mV vs. an Ag/AgCl reference electrode (LC-4B, BAS, IN, USA). The mobile phase was an aqueous potassium phosphate buffer (1.9 mM K_2HPO_4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) delivered at a constant flow of 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 60-90 min in order to monitor changes in electrode sensitivity, and sample concentrations were corrected accordingly.

2.3. Drugs

R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphtalenyl)methanone mesylate, WIN 55,212-2 (RBI, Natick, MA, USA), (6aR)-*trans*-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol, HU 210 (Tocris Cookson, UK) and {*N*-(piperidin-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-H-pyrazole-3-carboxamide}HCl, SR 141716A (Sanofi Research, France), were suspended in 0.3% TWEEN 80 in saline and administered intravenously in a volume of 1 ml/kg.

2.4. Statistics

Values are expressed as % 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 and two-way analysis of variance (ANOVA), with time as the repeated measure, were used to analyze 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 prefrontal cortical and hippocampal acetylcholine output and effects of vehicle on acetylcholine release

The overall mean ± S.E.M. baseline of acetylcholine in the dialysates from the prefrontal cortex and from the hippocampus was 65.4 ± 5.3 fmol/min ($n = 61$) and 32.5 ± 2.1 fmol/min ($n = 59$), respectively. Intravenous ad-

ministration of vehicle (0.3% of TWEEN 80 in saline solution) did not significantly affect basal acetylcholine output from prefrontal cortex, ($F(8,48) = 1.3$, NS) and hippocampus ($F(8,48) = 0.9$, NS) (Figs. 1 and 2).

3.2. Effect of WIN 55,212-2 on prefrontal cortical and hippocampal acetylcholine release

Fig. 1 (left panels) shows the effect of various doses of WIN 55,212-2 given intravenously on dialysate acetylcholine in the prefrontal cortex and in the hippocampus. WIN 55,212-2 significantly increased acetylcholine release at doses of 75 μg/kg ($F(9,36) = 3.11$, $P < 0.007$) and 150 μg/kg ($F(6,30) = 5.96$, $P < 0.0003$) in the hippocampus and at doses of 37.5 μg/kg ($F(8,40) = 3.05$, $P < 0.009$), 75 μg/kg ($F(9,36) = 3.7$, $P < 0.002$) and 150 μg/kg ($F(6,24) = 9.04$, $P < 0.0001$) in the prefrontal cortex. The dose of 10 μg/kg of WIN 55,212-2 did not significantly increase dialysate acetylcholine in the prefrontal cortex ($F(8,32) = 0.8$, NS) or hippocampus

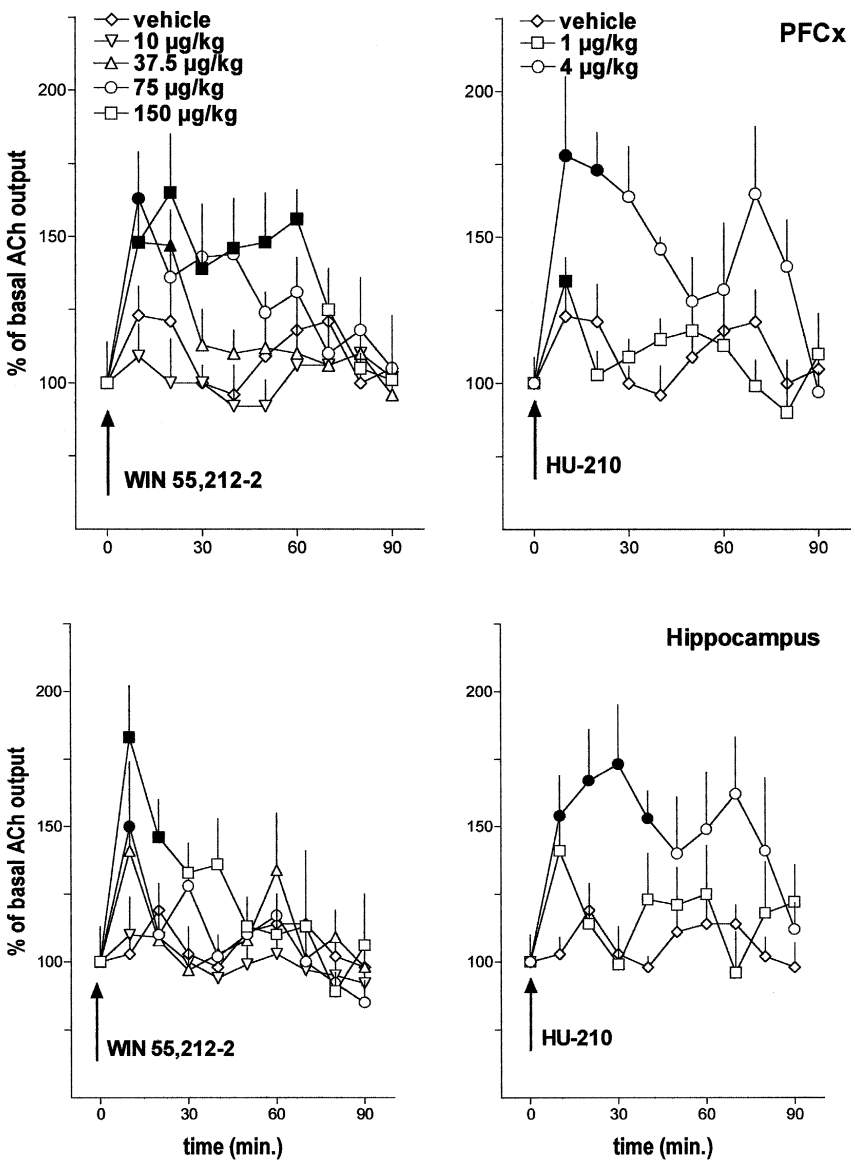

Fig. 1. Top left: effects of vehicle ($n = 7$) and WIN 55,212-2 (10, 37.5, 75 or 150 μg/kg i.v.) ($n = 5$, 6, 5 and 5, respectively) on prefrontal cortical acetylcholine release. Top right: effects of vehicle ($n = 7$) and HU 210 (1 or 4 μg/kg i.v.) ($n = 7$ and 5, respectively) on prefrontal cortical acetylcholine release. The effects of vehicle on cortical acetylcholine release in this panel, are from top left panel and are shown here for comparison. Bottom left: effects of vehicle ($n = 7$), WIN 55,212-2 (10, 37.5, 75 or 150 μg/kg i.v.) ($n = 5$, 6, 6 and 5, respectively) on hippocampal acetylcholine release. Bottom right: effects of vehicle ($n = 7$), HU 210 (1 or 4 μg/kg i.v.) ($n = 6$ and 5, respectively) on hippocampal acetylcholine release. The effects of vehicle on hippocampal acetylcholine release in this panel, are from bottom left panel and are shown here for comparison. 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).

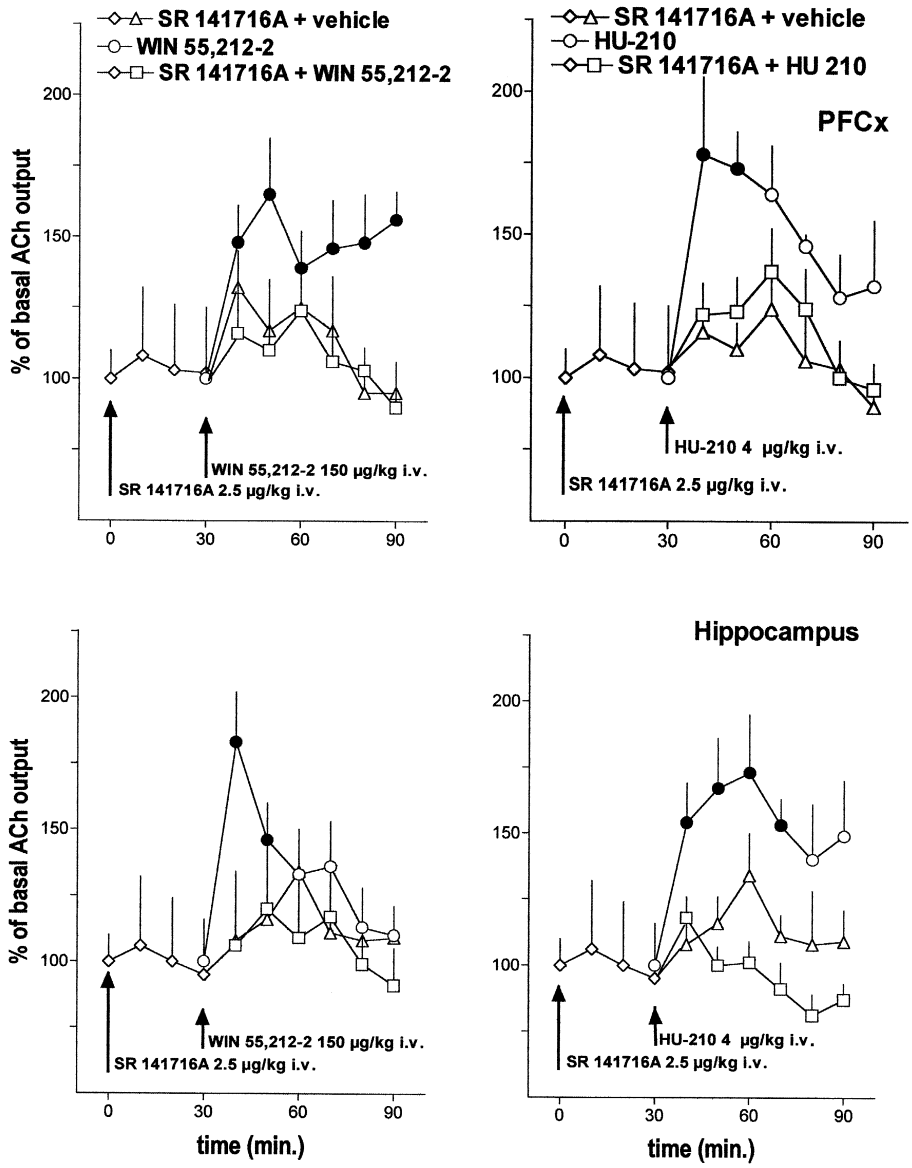

Fig. 2. Top left: effects of SR 141716A (2.5 μg/kg i.v.) on basal prefrontal cortical ($n = 4$) acetylcholine release and effects of SR 141716A (2.5 μg/kg i.v.) pretreatment on WIN 55,212-2 (150 μg/kg i.v.) induced increases on prefrontal cortical acetylcholine release ($n = 8$). The effects of WIN 55,212-2 (150 μg/kg i.v.) on cortical acetylcholine release, from Fig. 1 are shown here for comparison. Bottom left: effects of SR 141716A (2.5 μg/kg i.v.) on basal hippocampal ($n = 4$) acetylcholine release and effects of SR 141716A (2.5 μg/kg i.v.) pretreatment on WIN 55,212-2 (150 μg/kg i.v.) induced increases on hippocampal acetylcholine release ($n = 8$). The effects of WIN 55,212-2 (150 μg/kg i.v.) on hippocampal acetylcholine release from Fig. 1 are shown here for comparison. Top right: effects of SR 141716A (2.5 μg/kg i.v.) pretreatment on HU 210 (4 μg/kg i.v.) induced increases on prefrontal cortical acetylcholine release ($n = 9$). The effects of HU 210 (4 μg/kg i.v.) on cortical acetylcholine release, from Fig. 1 and those of SR 141716A (2.5 μg/kg i.v.) on basal cortical acetylcholine release from this figure, top left, are shown here for comparison. Bottom right: effects of SR 141716A (2.5 μg/kg i.v.) pre-treatment on HU 210 (4 μg/kg i.v.) induced increases on hippocampal acetylcholine release ($n = 7$). The effects of HU 210 (4 μg/kg i.v.) on hippocampal acetylcholine release from Fig. 1 and those of SR 141716A (2.5 μg/kg i.v.) on basal hippocampal acetylcholine release from this figure, bottom left, are shown here for comparison. 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).

($F(8,48) = 1.1$, NS). Fig. 3 shows the maximal changes of dialysate acetylcholine in the various groups, administered with WIN 55,212-2, as percentage of basal.

Two-way ANOVA revealed a significant effect of dose ($F(4,23) = 3.17$, $P < 0.03$) and time ($F(7,161) = 4.96$, $P < 0.0001$) in the prefrontal cortex and a significant effect of dose ($F(4,23) = 3.9$, $P < 0.01$) and time ($F(3,69) = 6.79$, $P < 0.0004$) in the hippocampus. Tukey's post-hoc analysis ($P < 0.05$) revealed that the effects of WIN 55,212-2 (150 μg/kg) were significantly different from

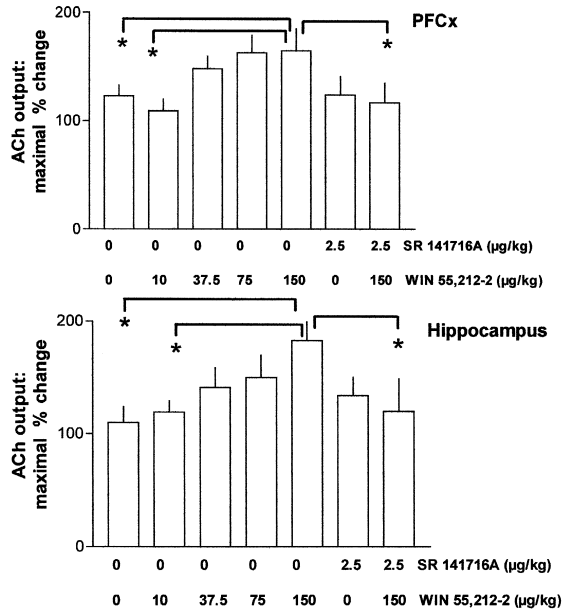

Fig. 3. Maximal % changes from basal values. Rats were pretreated with SR 141716A or saline and 30 min later with WIN 55,212-2. Columns indicate the maximal % change (±S.E.M.) of experimental groups; $^{*}p < 0.05$ at Tukey's post-hoc test with respect to the correspondent group (as indicated in the figure by the bracket).

those of WIN 55,212-2 (10 μg/kg) and vehicle both in the prefrontal cortex and hippocampus.

3.3. Effect of HU 210 on prefrontal cortical and hippocampal acetylcholine release

Fig. 1 (right panels) shows the effect of intravenous administration of HU 210 (1 and 4 μg/kg) on cortical and hippocampal acetylcholine output. At doses of 1 μg/kg ($F(9,54) = 2.89$, $P < 0.007$) and 4 μg/kg ($F(3,15) = 4.17$, $P < 0.016$) in the prefrontal cortex, and at the dose of 4 μg/kg ($F(9,36) = 3.41$, $P < 0.003$) in the hippocampus, HU 210 significantly changed acetylcholine output. The dose of 1 μg/kg of HU 210 did not significantly increase dialysate acetylcholine in the hippocampus ($F(9,45) = 1.5$, NS).

Two-way ANOVA revealed for the effects on prefrontal cortical and hippocampal acetylcholine release, significant effects of dose ($F(2,15) = 8.4$, $P < 0.003$) and ($F(2,15) = 5.17$, $P < 0.013$), respectively. Tukey's post-hoc analysis revealed that the increases after HU 210 4 μg/kg were significantly higher ($P < 0.05$) than those after HU 210 1 μg/kg in the prefrontal cortex but not in the hippocampus; also, Tukey's post-hoc test showed that the effects of HU 210 4 μg/kg on acetylcholine output both from the prefrontal cortex and from the hippocampus significantly differed from those of vehicle. Fig. 4 shows the maximal changes of dialysate acetylcholine in the various groups, administered with HU 210, as percentage of basal.

3.4. Effect of SR 141716A on prefrontal cortical and hippocampal acetylcholine release

Fig. 2 shows the effects of intravenous administration of SR 141716A (2.5 μg/kg) on cortical and hippocampal acetylcholine release. At this dose SR 141716A did not significantly affect basal acetylcholine release in the prefrontal cortex ($F(6,18) = 1.93$, NS) and hippocampus ($F(6,18) = 2.12$, NS).

3.5. Effect of SR 141716A on prefrontal cortical- and hippocampal-evoked increases of acetylcholine release by WIN 55-212,2 or HU 210

Figs. 2–4 show the effects of SR 141716A (2.5 μg/kg i.v.) administered 30 min before the administration of WIN 55,212-2 (150 μg/kg i.v.) or HU 210 (4 μg/kg i.v.) on cortical and hippocampal acetylcholine release. As shown in Fig. 2 (left panels) and Fig. 3, SR 141716A prevented the increase of prefrontal cortical ($F(6,36) = 1.99$, NS) and hippocampal ($F(5,30) = 1.58$, NS) acetylcholine release, respectively by WIN 55,212-2 (150 μg/kg). Two-way ANOVA revealed a significant effect of SR 141716A, in the prefrontal cortex ($F(1,10) = 7.02$, $P < 0.02$) and in the hippocampus ($F(1,8) = 5.5$, $P < 0.04$). Fig. 2 (right panels) and Fig. 4 show that SR 141716A (2.5 μg/kg

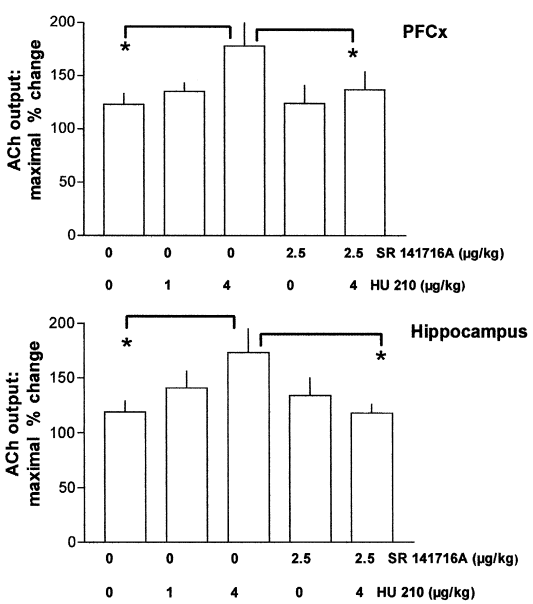

Fig. 4. Maximal % changes from basal values. Rats were pretreated with SR 141716A or saline and 30 min later with HU 210. Columns indicate the maximal % change (±S.E.M.) of experimental groups; $^{*}p < 0.05$ at Tukey's post-hoc test with respect to the correspondent group (as indicated in the figure by the bracket).

i.v.), administered 30 min before HU 210 (4 μg/kg i.v.) also prevented the increase of prefrontal ($F(1,12) = 6.98$, $P < 0.02$) and hippocampal ($F(1,8) = 17.15$, $P < 0.003$) acetylcholine release by HU 210. Two-way ANOVA revealed significant effects of SR 141716A in the prefrontal cortex ($F(1,10) = 7.02$, $P < 0.02$) and in the hippocampus ($F(1,8) = 5.5$, $P < 0.04$), respectively.

3.6. Behavioural effects of WIN 55,212-2, HU 210 and SR 141716A

The administration of WIN 55,212-2 at doses of 75 and 150 μg/kg induced a behavioural stimulation characterized by intense body grooming, wet-dog-shakes and sniffing at the air during collection of the first and second 10-min samples. During the third sample, rats appeared aroused and showed discontinuous behavioural activation. At the dose of 37.5 μg/kg, WIN 55,212-2 produced some sniffing and walking along the cage during the first 20 min after injection; the administration of 10 μg/kg of WIN 55,212-2 evoked only some sniffing during the first 10 min after injection. Administration of HU 210 4 μg/kg caused sniffing at the air, body grooming and some wet-dog-shakes during the first 10 min after injection; this behavioural activation after 4 μg/kg HU 210 lasted for at least 30 min, while at the dose of 1 μg/kg, HU 210 caused effects similar to the higher dose but less intense and limited to the 10 min of the first dialysis sample.

The intravenous administration of the cannabinoid CB_1 receptor antagonist, SR141716A, at the dose of 2.5 μg/kg did not produce overt behavioural effects. Pretreatment with SR 141716A (2.5 μg/kg) before WIN 55,212-2 (150 μg/kg) or HU 210 (4 μg/kg) reduced the intensity of the behavioural stimulation described above.

4. Discussion

The present study shows that intravenous administration of low doses of the cannabinoid agonists, WIN 55,212-2 and HU 210, increases acetylcholine release in the rat medial prefrontal cortex and hippocampus and that this effect is blocked by the cannabinoid receptor antagonist, SR 141716A, given at doses that do not significantly affect acetylcholine release from prefrontal cortex and hippocampus.

These results are at variance with those showing that cannabinoids inhibit acetylcholine release in vitro (Gifford and Ashby, 1996) and in vivo from the frontal cortex and hippocampus (Carta et al., 1998; Gessa et al., 1998a). However, the present study differed in many aspects from those of Carta et al., 1998 and Gessa et al., 1998a, such as the doses of WIN 55,212-2 (0.01–0.15 mg/kg vs. 5 and 10 mg/kg), the route of administration (intravenous vs. intraperitoneal), the anaesthesia utilized for implanting the probes (ketamine vs. equitesin) and finally the type of probes used (vertical concentric vs. transversal). Similar considerations apply to the differences between the present study and previous studies (Gessa et al., 1998a) in the effects of SR 141716A. Thus, the dose of SR 141716A utilized in the present study is at least 400 times lower (2.5 μg/kg i.v.) than those (1–3 mg/kg) utilized in the study of Gessa et al. (1998a). In view of the present observation of a stimulatory effect of low doses of cannabinoids on acetylcholine release and the partial agonist nature of SR 141716A (Bouaboula et al., 1997; Gifford and Ashby, 1996), the stimulation of acetylcholine release observed by Gessa et al. (1998a) might be the result of stimulation of cannabinoid receptors by SR 141716A.

In the present study, the effect of cannabinoids on acetylcholine release was compared in each rat in the medial prefrontal cortex and dorsal hippocampus. At the lower doses tested, cannabinoids increased acetylcholine release to a similar extent in the hippocampus and in the prefrontal cortex, an observation consistent with the idea that the basal forebrain cholinergic complex (Schwaber et al., 1987) acts as a functional unit (Acquas et al., 1996).

The mechanism by which WIN 55,212-2 and HU 210 increase cortical and hippocampal acetylcholine release remains to be elucidated. Dopamine release in the nucleus accumbens is thought to control acetylcholine release in the cortex (Casamenti et al., 1985; Moore et al., 1999). Since cannabinoids in the range of doses used in the present study stimulate dopamine release in the shell of the nucleus accumbens (Tanda et al., 1997) and the firing of dopaminergic neurons in the ventral tegmental area (Gessa et al., 1998b), one might suggest that release of acetylcholine by low doses of cannabinoids is indirectly related to activation of dopamine through its effects on motor activity and arousal. In this regard, it is notable that pretreatment with SR 141716A not only reduces the increase of acetylcholine release in the prefrontal cortex and hippocampus, but also partially reduces the behavioural stimulation by cannabiniods.

The present observations further emphasize the complexity of the relationship between behavioural changes induced by cannabinoids and acetylcholine transmission. In fact, not only the memory impairment induced by cannabinoids might be unrelated to a reduction of acetylcholine release (Lichtman and Martin, 1996; Presburger and Robinson, 1999), but it is also possible that changes in acetylcholine release after low doses of these compounds are the consequence of the affective and motivational effects of cannabinoids rather than the cause.

The present study shows that low, intravenous doses of cannabinoids stimulate acetylcholine release in the prefrontal cortex and in the hippocampus. As our conditions are more likely to mimic those of cannabinoid exposure through smoke in humans than those utilized in previous studies where high doses of cannabinoids were given i.p. (Carta et al., 1998; Gessa et al., 1998a), it is suggested that the changes in acetylcholine release observed here might

reflect the changes taking place in humans smoking cannabis derivatives (marijuana and hashish). If this argument is correct, one would predict that exposure to cannabis in humans is associated to an increase rather than a decrease in brain acetylcholine release. However, the relationship between these changes and the cognitive effects of cannabinoids is at the moment obscure.

Acknowledgements

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