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Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR 141716A

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Abstract

Two synthetic cannabinoids, WIN 55,212-2 {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinylmethyl]pyrol [1,2,3-*de*]-1,4-benzoxazin-6-yl)(1-naphthalenyl)methanone monomethanesulfonate} (5.0 and 10 mg/kg i.p.) and CP 55,940 {[1*a*,2-(*R*)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol} {[1*a*,2-(*R*)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol} (0.5 and 1.0 mg/kg i.p.), inhibited acetylcholine release in the rat hippocampus. The inhibition was prevented by the cannabinoid receptor antagonist, SR 141716A {*N*-(piperidin-1-yl)-5-(4- chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide} HCl, at the dose of 0.1 mg/kg i.p. Higher doses of SR 141716A (1.0 and 3.0 mg/kg i.p.) themselves increased hippocampal acetylcholine release, suggesting that acetylcholine output is tonically inhibited by endogenous cannabinoids. The results also suggest that the negative effects of marijuana on learning and memory may depend on cannabinoid receptor-mediated inhibition of acetylcholine release.

Keywords: Hippocampus; Acetylcholine; Cannabinoid

Δ^9-Tetrahydrocannabinol {(−)-*trans*-(6*aR*,10*aR*)-6*a*,7,8,10*a*-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol}, the psychoactive principle of marijuana, impairs 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, two structurally distinct synthetic cannabinoid receptor agonists, WIN 55,212-2 {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinylmethyl]pyrol [1,2,3-*de*]-1,4-benzoxazin-6-yl)(1-naphthalenyl)methanone monomethanesulfonate} and CP 55,940 {[1*a*,2-(*R*)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol}, were found to impair working memory in rats, not only after systemic but also after intrahippocampal administration, suggesting that these effects are mediated by cannabinoid receptors densely located in this area (Lichtman et al., 1995).

In order to determine if cannabinoid receptors might control the septo-hippocampal cholinergic system, that is considered to play a crucial role in learning and memory processes, the effect of WIN 55,212-2, CP 55,940 and of the potent and selective cannabinoid receptor antagonist SR 141716A {*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide} HCl (Rinaldi-Carmona et al., 1994) on the extracellular concentration of acetylcholine in the hippocampus of conscious, freely moving rats was measured by microdialysis.

Male Sprague-Dawley rats (200–250 g) were implanted in the hippocampus with a transversal microdialysis probe as previously described (Imperato et al., 1992). Twenty-four hours after surgery, the probes were perfused with Ringer solution containing (mM) KCl 3.0, NaCl 125, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $NaHCO_3$ 23, potassium phosphate buffer 1.5 pH 7.3 and neostigmine 0.1 mM at a constant rate of 2 ml/min. Samples (40 ml) were collected every 20 min. Acetylcholine was measured by high performance liquid chromatography (HPLC) and electrochemical detection. The mean concentration of acetylcholine in the last 3 pre-drug samples, obtained after 1-h perfusion, was considered as the baseline value; post-treatment values were expressed as mean ± S.E.M. percent variation of baseline value.

The average baseline extracellular acetylcholine concentration (± S.E.M.) in the hippocampus in all of the animals

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tested was 1.68 ± 0.17 pmol/sample. There was no difference in baseline level between experimental groups (5–6 rats for each treatment).

The intraperitoneal administration of WIN 55,212-2 (5.0 and 10 mg/kg) and CP 55,940 (0.5 and 1.0 mg/kg) produced a dose-related inhibition of acetylcholine release (Fig. 1). A maximal inhibition of 55 and 50% occurred within 60 min with the dose of 1.0 and 10 mg/kg of CP 55,940 and WIN 55,212-2, respectively, and was still present at 180 min. The effect of CP 55,940 and WIN 55,212-2 was prevented by SR 141716A, at the dose of 0.1 mg/kg, itself ineffective to modify acetylcholine release. Most interestingly, SR 141716A alone, at the doses of 1.0 and 3.0 mg/kg, increased acetylcholine release by 30 and 45% within 20 min, respectively. The increase was still present at 180 min.

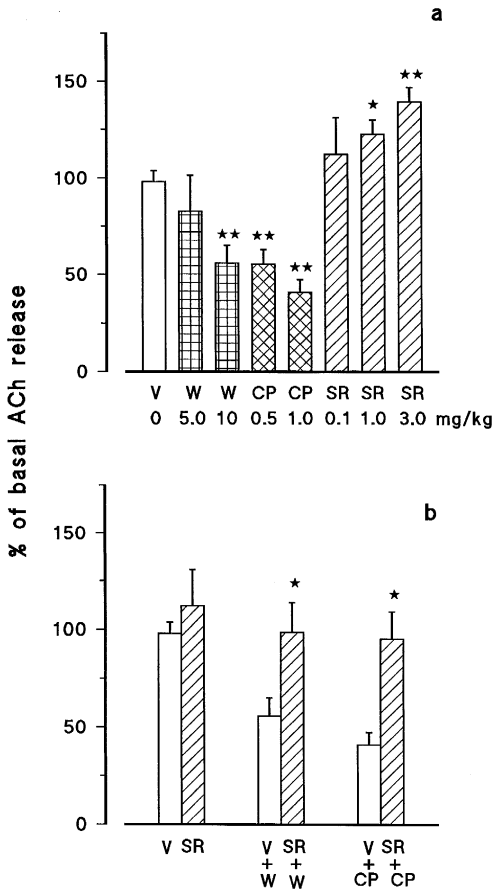

Fig. 1. (a) Effect of WIN 55,212-2 and CP 55,940 on hippocampal acetylcholine release in the rat. (b) Reversal of this effect by SR 141716A. Data were obtained from samples collected 60 min after treatment, at the time of peak effect. Each value, calculated as percent of baseline concentrations, represents the mean ± S.E.M. for 4–11 rats per group. In the experiments of panel b, SR 141716A (0.1 mg/kg i.p.) or vehicle was given 5 min before WIN 55,212-2 or CP 55,940 (10 and 1 mg/kg i.p., respectively). SR 141716A and CP 55,940 were dissolved in Tween-80 and saline; WIN 55,212-2 was dissolved in Cremophor EL and saline. V, vehicle; W, WIN 55,212-2; CP, CP 55,940; SR, SR 141716A. * $P < 0.05$, ** $P < 0.01$ with respect to the vehicle-treated group (a) and to the corresponding vehicle pre-treated group (b). One-way analysis of variance followed by Dunnett's test.

The inhibitory effect of WIN 55,212-2 and CP 55,940 on hippocampal acetylcholine release is most likely mediated through cannabinoid receptors, as it is produced by two structurally distinct compounds, with comparative potency consistent with their affinity for cannabinoid receptors (Compton et al., 1996) and is suppressed by the specific cannabinoid receptor antagonist, SR 141716A. On the other hand, the finding that SR 141716A per se increased acetylcholine release suggests that this compound may antagonize endogenous cannabinoids controlling cholinergic transmission in this area.

Our results suggest that the negative effects of cannabinoids on cognitive processes are mediated through cannabinoid receptors regulating acetylcholine transmission in the hippocampus and that cannabinoid antagonists may offer potential treatment for cognitive deficits.

Results of experiments in progress in our laboratory show that acetylcholine output increases following intrahippocampal SR 141716A perfusion, and thus suggest that cannabinoid receptors controlling acetylcholine output are located in the hippocampus itself.

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