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SPECIAL REPORT

Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice

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Incubation of rat hippocampal slices in the presence of the synthetic cannabinoid (–)-11-OH-Δ^8-dimethylheptyl tetrahydrocannabinol (HU-210) (100 nM) prevented the induction of long-term potentiation (LTP). Slices co-incubated with both HU-210 (100 nM) and the cannabinoid antagonist, SR141716A (100 nM), exhibited tetanically induced LTP, comparable to control slices. Intriguingly, co-incubation with HU-210 and SR141716A prevented the induction of the early, short-term phase of LTP.

Keywords: Cannabinoid; long-term potentiation; hippocampus; SR141716A

Introduction Long-term potentiation (LTP) describes a form of use-dependent synaptic plasticity that is a proposed model for learning and memory formation (Bliss & Collingridge, 1993). High affinity cannabinoid receptor binding sites are found in those brain regions, i.e. hippocampus and cortex, which exhibit LTP (Herkenham *et al.*, 1991). The memory deficits induced by cannabinoid administration have been recognised for many years (Miller & Braconnier, 1983) and application of Δ^9-tetrahydrocannabinol, the major component of marihuana has also been shown to prevent induction of LTP (Nowicky *et al.*, 1987). We have previously shown that application of the psychoactive synthetic cannabinoid, (–)-11-OH-Δ^8-dimethylheptyl-tetrahydrocannabinol (HU-210) prevents the formation of tetanically induced LTP in a stereospecific manner (Collins *et al.*, 1994). Here we have investigated the effect of SR141716A (N-(piperidin-1-yl)-5-(-4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride), a highly selective and a competitive ligand for CB_1 cannabinoid receptors (Rinaldi-Carmona *et al.*, 1994), on the blockade of LTP by HU-210.

Methods For all experiments the drugs were mixed with two parts of Tween 80 by weight and then dispersed in standard perfusion medium. Transverse rat hippocampal slices (400 μm thick) were prepared and pre-incubated for 60–90 min with 100 nM HU-210, with 100 nM SR141716A, with 100 nM SR141716A plus 100 nM HU-210, or with normal medium containing an equivalent concentration of Tween 80. The concentration of SR141716A used was selected as 100 nM since this is in excess of concentrations shown to displace and reverse the effects of cannabinoids in rat brain synaptosomal membrane preparations (Rinaldi-Carmona *et al.*, 1994). The CA3 region was removed and the slices transferred to a standard interface chamber where they were constantly perfused (1 ml min^{-1}) with standard medium at 30°C (Collins *et al.*, 1994).

The Schaffer collateral commissural fibre pathway was stimulated at a frequency of 0.033 Hz and extracellular field excitatory postsynaptic potentials (field e.p.s.ps) were recorded from the CA1 region. Synaptic LTP was induced by the application of 1 or 2 bursts of high frequency stimuli (tetani) at a frequency of 100 Hz for 500 ms at 3 times the test stimulus strength.

Comparison of pre- and post-tetanic field e.p.s.p. amplitudes were made by Student's two-tailed, paired *t* test. *P* values of less than 0.05 were regarded as significant.

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Results Tetanic stimulation of slices incubated in control medium evoked a rapidly forming and long-lasting potentiation of the synaptic response (mean field e.p.s.p. amplitude ± standard error was 187 ± 16% of control at 10 min post tetanus, 158 ± 18% at 50 min post tetanus, $P < 0.001$, $n = 6$, see Figure 1).

As previously reported (Collins *et al.*, 1994), incubation in the presence of 100 nM HU-210 prevented the formation of tetanically induced LTP, the field e.p.s.p. amplitude returning to baseline levels within approximately 10 min after tetanus application (mean field e.p.s.p. amplitude was 103 ± 17% at 10 min, 75 ± 21% at 50 min post tetanus, not significant, $n = 5$, see Figure 1).

In slices incubated in the presence of the cannabinoid receptor antagonist, SR141716A, the pre-tetanus responses were generally smaller than those recorded in control conditions and were more excitable, readily generating population spikes and taking a greater time to stabilise prior to tetanus application. In all 5 slices tetanic stimulation evoked robust LTP with a comparable time course to that recorded in control slices, although the magnitude of the potentiation was slightly reduced (mean field e.p.s.p. amplitude was 137 ± 9% at 10 min, 140 ± 5% at 50 min post tetanus, $P < 0.01$, $n = 5$, data not shown).

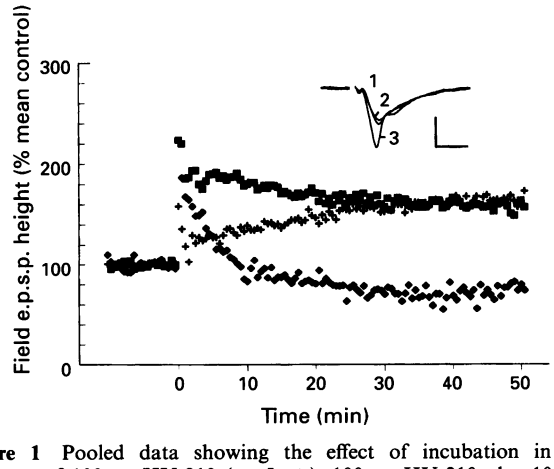

Figure 1 Pooled data showing the effect of incubation in the presence of 100 nM HU-210 ($n = 5$, ◆), 100 nM HU-210 plus 100 nM SR141716A ($n = 5$, +) or control medium ($n = 6$, ■). Tetanic stimulation was delivered to all slices at time 0. The inset traces illustrate responses from a representative slice incubated in HU-210 plus SR141716A showing control responses (1) and responses taken 10 min (2, the slightly larger response) and 50 min (3) after tetanic stimulation. The scale bar represents 1 mV by 10 ms.

When slices were co-incubated with HU-210 (100 nM) and SR141716A (100 nM) we found that HU-210-induced inhibition of LTP no longer occurred. Interestingly, in 5 out of the 6 trials, there was a marked loss of short-term potentiation (STP) so that following a brief, presumed post-tetanic, potentiation the response returned to control size and then gradually potentiated over the trial period (mean field e.p.s.p. amplitude was $131 \pm 6\%$ at 10 min, $163 \pm 8\%$ at 50 min post tetanus, $P = 0.001$, $n = 5$, see Figure 1).

Discussion The results show that the cannabinoid receptor antagonist SR141716A can prevent the effects of the synthetic psychoactive cannabinoid, HU-210, on LTP. Intriguingly though, a phase of short-term potentiation lasting up to 20 min after the tetanus remained blocked.

This is, to our knowledge, the first instance of tetanic stimulation (as opposed to drug perfusion) evoking a late phase of LTP in the absence of STP. Blockade of STP does not seem to be caused by an action of the antagonist alone, since although slices incubated in SR141716A exhibited a smaller and more excitable pre-tetanus response, they still showed robust synaptic potentiation (STP and LTP) which displayed a comparable time course to that evoked in slices incubated in Tween 80 alone.

The actions of SR141716A (and HU-210) on specific components of the synaptic response clearly need to be elucidated, but the observation that effects of HU-210 are both stereoselective (Collins *et al.*, 1994) and prevented by a known CB_1 cannabinoid receptor antagonist, strengthen the conclusion that HU-210 inhibits LTP via an action at a specific cannabinoid receptor.

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