Original Article

Repeated administration of cannabinoid receptor agonist and antagonist impairs short and long term plasticity of rat's dentate gyrus *in vivo*

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Abstract

Introduction: The effects of cannabinoids (CBs) on synaptic plasticity of hippocampal dentate gyrus neurons have been shown in numerous studies. However, the effect of repeated exposure to cannabinoids on hippocampal function is not fully understood. In this study, using field potential recording, we investigated the effect of repeated administration of the nonselective CB receptor agonist WIN55212-2, and the CB1 receptor antagonist AM251, on both short- and long-term synaptic plasticity in dentate gyrus (DG) of hippocampus.

Materials and Methods: Drugs were administered three times daily for seven consecutive days into lateral ventricle of rats. Short term synaptic plasticity was assessed by measuring paired-pulse index (PPI) in DG neurons after stimulation of perforant pathway. Long-term plasticity was assessed through measurement of both population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP) slope after high frequency stimulation (HFS) of DG neurons.

Results: Repeated administration of WIN55212-2 not only significantly decreased PPI in 20, 30 and 50 ms intervals but also blocked LTP. This effect was reversed by pretreatment of rats with CB1 receptor antagonist AM251. Moreover, AM251 by itself increased PPI in 10 and 20 ms interval stimulations, but had no effect on HFS-induced PS amplitude and fEPSP slope.

Conclusion: These results suggest that repeated administration of cannabinoids could impair short term and long term synaptic plasticity that may be due to desensitization of cannabinoid receptors and/or changes in synaptic spine density of hippocampus which leads to alteration in short and long term memories that remains to be elucidated.

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Introduction

Cannabinoids can affect a variety of cognitive and performance tasks, including learning, memory, and attention (Hampson and Deadwyler, 1999). A well-known cellular effect of cannabinoids is the presynaptic inhibition of both GABAergic and glutamatergic transmission throughout the brain (Hohmann *et al.*, 2005). In the hippocampus, this effect

appears to be the primary cause by which cannabinoids acutely disrupt neuronal network activity (Hajos *et al.*, 2000). Immunocytochemical and electrophysiological studies revealed that in the hippocampus CB1 receptors are expressed on axon terminals of GABAergic inhibitory interneurons and activation of these receptors decreases GABA release (Hajos *et al.*, 2000, Andó *et al.*, 2012). On the other hand, *in vitro* studies demonstrated that acute cannabinoid administration suppresses excitatory synapses on stratum radiatum interneurons in the hippocampus (Edwards *et al.*, 2012). However, changes of hippocampal function after repeated exposure to cannabinoids is poorly understood (Ameri *et al.*, 1999, Hoffman *et al.*, 2007). In the present study, the effect of repeated intracerebroventricular (i.c.v.) administration of nonselective cannabinoid receptor agonist WIN55212-2 as well as cannabinoid CB1 antagonist AM251 on paired pulse index (which reflects short term plasticity) was investigated. In addition, for evaluation of the effect of these compounds on long term plasticity, long term potentiation (LTP), PS amplitude and fEPSP slope of DG area have also been evaluated.

Materials and methods

Male wistar rats were housed three per cage in a room with controlled temperature (22 ± 2°C) and 12h-12h light/dark cycle. Food and water were provided *ad libitum*. All drugs were obtained from SIGMA-ALDRICH (St. Louis, MO). The nonselective cannabinoid receptor agonist WIN55212-2, the cannabinoid CB1 receptor antagonist AM251 or their vehicle (DMSO 20% in saline) were administered through intracerebroventricular (i.c.v.; 5µL/rat) injection. The experiments were conducted in accordance with the Guide for Care and Use of Laboratory animals published by the United States National Institute of Health (NIH Publication No. 52-23, Revised 1985).

For i.c.v. administration of drugs, rats were anesthetized by i.p. injection of a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg) and were placed in stereotaxic apparatus. The stereotaxic coordinates for injection into right lateral ventricle were according to

the atlas of Paxinos and Watson (AP = -1 from bregma; ML = 1.6I; DV = 3.6 mm from the skull surface) (Paxinos and Watson, 2007) and a 23-gauge cannula was then lowered one mm above the injection site and fixed by dental cement. After one week of recovery period, the drugs or their vehicle were injected three times a day for seven consecutive days (Hoffman *et al.*, 2007, Candelaria-Cook and Hamilton, 2014). Rats divided into four distinct groups. At the time of drug delivery, a 30-gauge infusion cannula was tightly fitted into the guides. Infusions were carried out over 60 s and the cannula was left in place for 60 additional seconds to minimize backflow. Three groups received either vehicle (control group), WIN55212-2 (25 µg/rat) or AM251 (0.5 µg/rat). One group received co-administration of AM251 (0.5 µg/rat) and WIN55212-2 (25 µg/rat). After seven days treatment protocol, rats were anesthetized by i.p. injection of 1.5 g/kg urethane and placed in a stereotaxic device. Supplementary injections of urethane (0.2 – 0.5 g/kg) were given when necessary to ensure full anesthesia. A heating pad was used to maintain the temperature of the animals at 36.5 ± 0.5 °C. Small holes were drilled in the skull at the positions of the stimulating and recording electrodes. The exposed cortex was kept moist by the application of warm mineral oil. The bipolar stainless steel recording and stimulating electrodes (0.125 mm diameter, Advent, UK), in which the tips were 1 mm apart, were positioned in the granular cells of DG (AP = −3.8; ML = 2.3; DV = 2.7–3.2 mm from the skull surface) and perforant pathway (AP = −8.1; ML = 4.3; DV = 3.2 mm from the skull surface), respectively according to the atlas of Paxinos and Watson (Paxinos and Watson, 2007). Implantation of electrodes in the correct position was determined by histological verifications. The test stimuli were delivered at 0.1 Hz (Kim *et al.*, 2005) to the perforant pathway every 10 s with constant current stimuli. Stimulation intensity was adjusted to elicit a maximal field PS and fEPSP. The PS amplitude was measured as the difference in voltage between the peak of the first positive wave and the peak of the first negative deflection and the fEPSP slope was measured as the maximum slope between the initial point of fEPSP and the first positive wave in order to measure synaptic efficacy. PS and field EPSPs were evoked in the DG

region using 0.1 Hz stimulation. Baseline recordings were taken at least 30 min prior to each experiment.

As previously reported from our laboratory (Lashgari *et al.*, 2007), extracellular field potentials were amplified (×1000); band pass filtered between 1 Hz and 10 KHz, digitized and recorded with the DAM 80 differential amplifier (WPI, USA) and data were analyzed using a home-made software. In order to determine the synaptic changes of DG neurons and its inhibitory interneurons, after ensuring a steady state baseline response, LTP was induced using a high-frequency stimuli protocols of 200 Hz (10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval) at a stimulus intensity that evoked a PS amplitude and field EPSP slope of approximately 80% of maximum response. All potentials employed as baseline before and after high frequency stimuli were evoked at a stimulus intensity which produced 40% of this maximum. Both fEPSP and PS were recorded every 5 min for the periods of 120 min after the high frequency stimuli in order to determine any changes in the synaptic response of DG neurons. For each time-point, 10 consecutive evoked responses were averaged at 10 s stimulus interval (Lashgari *et al.*, 2006).

For the short term plasticity assessment paired pulse depression/facilitation was measured by delivering four consecutive evoked responses of paired pulses at 10, 20, 30 and 50 ms inter-stimulus intervals to the perforant pathway at a frequency of 0.1 Hz (10s interval). Population spike amplitude ratio (PS amplitude2 / PS amplitude1 × 100) and paired pulse index (PPI) were then measured at different inter-stimulus intervals and compared with the control group.

In order to assess significant difference between experimental groups, one-way analysis of variance (ANOVA) followed by Dunnett's post test was used for comparison of PPI between the treated groups. In order to compare replicated means of fEPSP and PS amplitude between treated groups in different time points, two-way repeated measure ANOVA followed by Bonferroni's post test was used. The significance level was set at $p < 0.05$. Results are expressed as mean ± SEM.

Results

The effect of repeated i.c.v. administration of vehicle, WIN55212-2, AM251 and co-administration of AM251 and WIN55212-2 on paired pulse index in hippocampal DG neurons is shown in Fig.1. A significant increase in paired pulse index was observed in rats treated with AM251 (0.5 µg) especially in shorter inter-stimulus intervals which was significantly different at 10ms and 20ms compared to their respective control group ($p<0.01$ and $p<0.05$, respectively) which represents paired pulse facilitation. In contrast, a decrease in paired-pulse index was observed in the WIN55212-2 treated group which was significantly different at inter-stimulus intervals of 20, 30 and 50ms compared to the control group ($p<0.05$). However, co-administration of AM251 and WIN55212-2 had no significant effect on all PPIs in comparison with the control group. A typical example of averaged extracellular evoked responses elicited from the DG cells with paired pulse (10, 20, 30 and 50 ms inter-stimulus intervals) is illustrated in the inset of Fig. 1.

The effect of repeated i.c.v. administration of vehicle, WIN55212-2, AM251 and co-administration of AM251 and WIN55212-2 on LTP induction of DG neurons is shown in Fig. 2. Two-way repeated measure ANOVA revealed a significant effect of each treatment on PS-LTP amplitude. [$F(3,368)=4.938$, $p = 0.013$; Fig. 2A]. In fact, AM251 and co-administration of AM251 and WIN55212-2 did not produce significant change in PS amplitude after HFS application compared to the control group. But repeated i.c.v. administration of WIN55212-2 blocked PS-LTP of DG neurons. Further analysis using Bonferroni's test showed significant decrease in PS amplitude of the WIN55212-2 treated group compared to the vehicle group ($p < 0.05$) after HFS application. As shown in Fig. 2B, application of WIN55212-2 also blocked fEPSP-LTP in DG neurons ($p <0.05$). But AM251 and co-administration of AM251 and WIN55212-2 had no significant change on fEPSP-LTP in comparison with the control group.

Discussion

The precise mode of action of cannabinoids on the hippocampal networks is still controversial. It has been
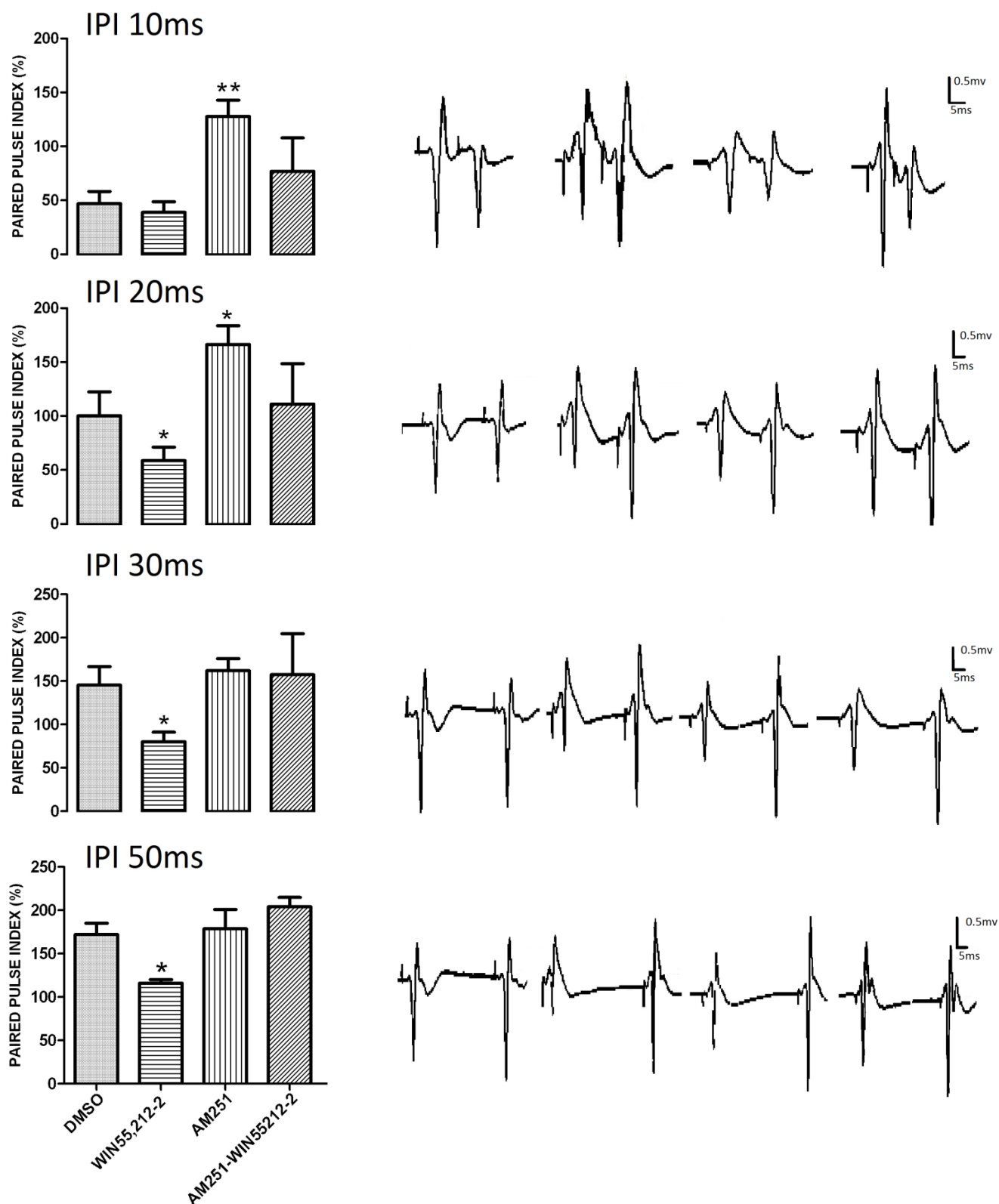
Fig. 1: The effect of repeated i.c.v. administration of vehicle (DMSO), WIN55212-2, AM251 and co-administration of WIN55212-2 and AM251 on paired pulse index (PPI) in dentate gyrus of the hippocampus at the population spike amplitude ratio in 10, 20, 30 and 50ms interstimulus intervals. The corresponding representative recordings are presented next to related graph. Values are percentage of mean PS2/PS1±S.E.M.

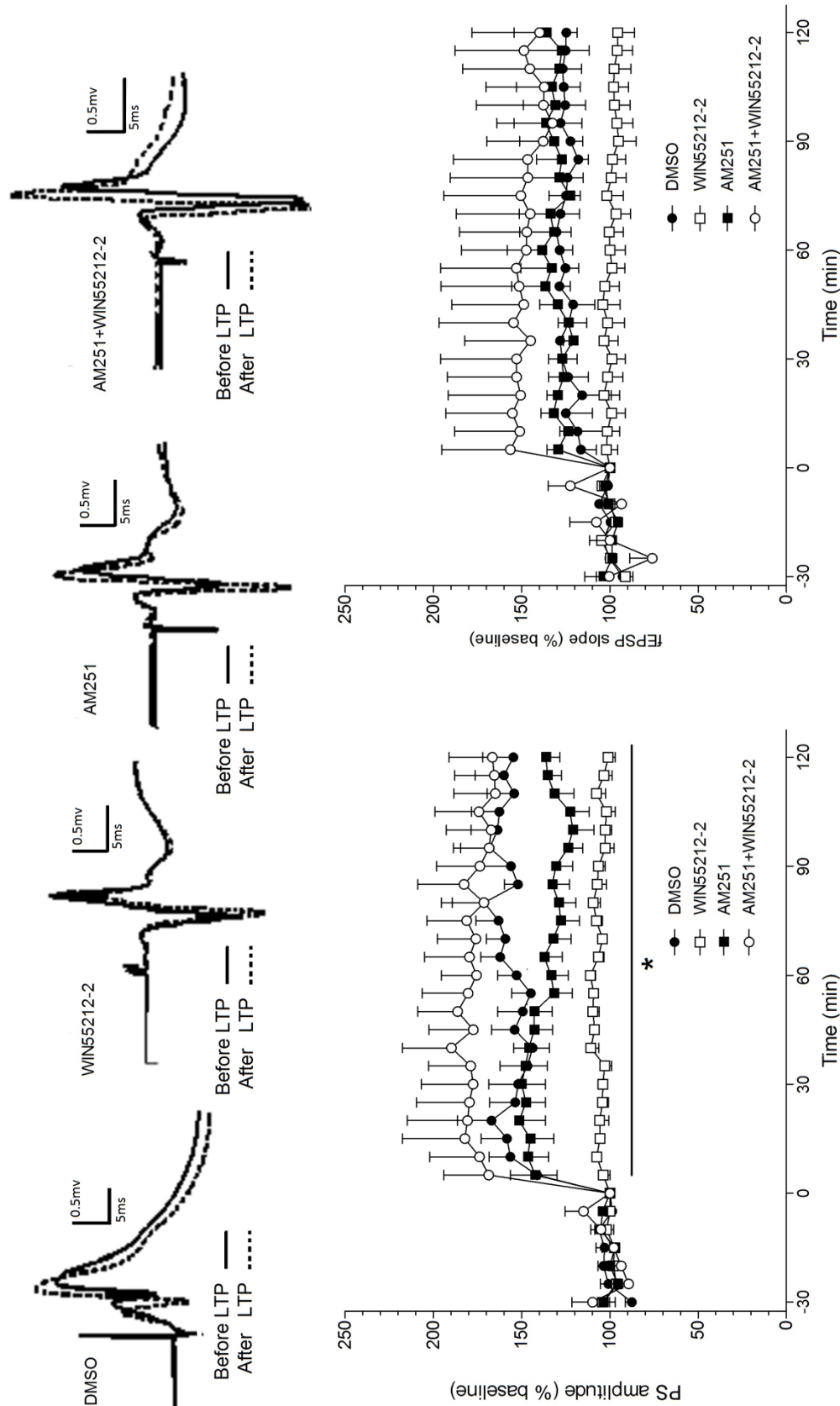*p <0.05, **p<0.01 compared to control group (N=6)

Fig 2: The effects of repeated i.c.v. injections of vehicle, WIN55212-2, AM251 and co-administration of WIN55212-2 and AM251 on HFS-induced PS-LTP amplitude (A) and EPSP-LTP slope (B) in the dentate gyrus of the hippocampus. The corresponding representative recordings are shown above the graph. Data are plotted as an average of the percentage change from baseline responses. Values are mean ± S.E.M.

shown that acute administration of cannabinoids reduces glutamate release and impairs hippocampal LTP and suppresses excitatory synaptic activity in the hippocampus (Misner and Sullivan, 1999, Katona *et al.*, 2006). On the other hand, several authors suggested that modulation of GABAergic system is an important mechanism of cannabinoid action on LTP (Collins *et al.*, 1995). CB1 receptors are expressed by GABAergic inhibitory interneurons in the hippocampus and modulation of GABAergic systems is an important component of their effects (Paton *et al.*, 1998). On the other hand, there are also reports indicating existence of CB1 receptors on excitatory presynaptic terminals as well (Domenici *et al.*, 2006). Activation of the CB1 receptors on GABAergic interneurons is thought to control hippocampal oscillations (Hajos *et al.*, 2000) and endogenous cannabinoids may modulate the synchronous spiking of hippocampal cells. The data of the present study is consistent with the idea of inhibitory role of CB1 receptors on GABA release. Our results showed a decreasing effect of cannabinoid receptor agonist WIN55212-2 on paired-pulse index at 20, 30 and 50 ms inter-stimulus intervals. This pattern of stimulation is related to paired-pulse inhibition, which is characterized by decrease in the amount of neurotransmitter release in response to the second stimulus (Atluri and Regehr, 1996). On the other hand, an increasing effect of the cannabinoid CB1 receptor antagonist AM251 on paired-pulse index was observed at 10 ms and 20 ms inter-stimulus intervals. This pattern of stimulation is related to paired-pulse facilitation, which is characterized by increase in the amount of neurotransmitter release in response to the second stimulus (Lashgari *et al.*, 2007). The data for WIN55212-2 is in line with report of Kirby et al. which reported that this drug decreased paired pulse facilitation in dentate gyrus *in vitro* (Kirby *et al.*, 1995). Moreover, the suppressive effect of repeated administration of WIN55212-2 on paired-pulse index is likely due to a reduction in the sensitivity of cannabinoid receptors expressed on GABAergic interneurons, which increase the effect of these interneurons upon postsynaptic glutamatergic neurons leading to augmentation of paired pulse inhibition. In contrast, repeated administration of CB1 receptor antagonist AM251 may produce an increase in the sensitivity of cannabinoid receptor to endogenous ligands which results in further inhibitory effect of cannabinoids on GABAergic interneurons (disinhibition) that eventually augments glutamate activation and leading to paired-pulse facilitation.

In the other part of this study repeated administration of WIN55212-2 significantly attenuated the HFS-induced potentiation in PS amplitude compared to the control group. This effect of WIN55212-2 was blocked by co-administration of AM251 and WIN55212-2 which implies a receptor-mediated response. However, repeated administration of AM251 did not produce significant changes in PS amplitude compared to control group. It seems that, down-regulation of CB1 receptors by repeated administration of CB1 agonists plays a major role in both short- and long-term synaptic plasticity in the hippocampus as Trezza et al. have reported (Trezza *et al.*, 2008). Such down-regulation of CB1 receptors has been previously observed after chronic treatment with the synthetic cannabinoid HU210 (Dalton and Zavitsanou, 2010). The reduction in CB1 receptor binding after repeated cannabinoid exposure in the hippocampus is likely related to development of tolerance for memory effects (Gonzalez *et al.*, 2005).

In conclusion, the results of the present study suggest that, in the hippocampus, cannabinoid receptor activation mainly modifies synapses onto GABAergic interneurons located in DG. Down-regulation of cannabinoid receptors after repeated administration of exogenous cannabinoid may attenuate such an inhibitory effect on GABA release and eventually leads to inhibition of both short- and long-term plasticity. In contrast, up-regulation of cannabinoid receptors following repeated administration of cannabinoid antagonists augments the effect of endocannabinoids on GABAergic inhibitory interneurons leading to improvement of short-term and long-term plasticity. However, the involvement of inhibitory effects of CBs on glutamate release in the hippocampus cannot be ruled out in this process which elucidated in future studies.

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Conflict of interest

All authors declared that there is no conflict of interest.

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