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Interactive effects of AM251 and baclofen on synaptic plasticity in the rat dentate gyrus

Masoumeh Nazari, Alireza Komaki *, Iraj Salehi, Abdolrahman Sarihi, Siamak Shahidi, Hamidreza Komaki, Ahmad Ganji

Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

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ABSTRACT

Long-term potentiation (LTP), a form of synaptic plasticity, is considered to be a critical cellular mechanism that underlies learning and memory. Cannabinoid CB_1 and metabotropic $GABA_B$ receptors display similar pharmacological effects and co-localize in certain brain regions. In this study, we examined the effects of co-administration of the CB_1 and $GABA_B$ antagonists AM251 and baclofen, respectively, on LTP induction in the rat dentate gyrus (DG).

Male Wistar rats were anesthetized with urethane. A stimulating electrode was placed in the lateral perforant path (PP), and a bipolar recording electrode was inserted into the DG until maximal field excitatory postsynaptic potentials (fEPSPs) were observed. LTP was induced in the hippocampal area by high-frequency stimulation (HFS) of the PP. fEPSPs and population spikes (PS) were recorded at 5, 30, and 60 min after HFS in order to measure changes in the synaptic responses of DG neurons.

Our results showed that HFS coupled with administration of AM251 and baclofen increased both PS amplitude and fEPSP slope. Furthermore, co-administration of AM251 and baclofen elicited greater increases in PS amplitude and fEPSP slope.

The results of the present study suggest that CB_1 receptor activation in the hippocampus mainly modifies synapses onto GABAergic interneurons located in the DG. Our results further suggest that, when AM251 and baclofen are administered simultaneously, AM251 can alter GABA release and thereby augment LTP through $GABA_B$ receptors. These results suggest that functional crosstalk between cannabinoid and GABA receptors regulates hippocampal synaptic plasticity.

1. Introduction

The hippocampus has long been considered important for learning and memory storage (Bermúdez-Rattoni, 2007; Shew et al., 2000; Bliss and Lømo, 1973; Lynch et al., 1979). It is generally believed that most information is stored at synapses in the form of modification in synaptic efficiency. In particular, two forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), have been broadly considered in the pursuit of understanding the molecular and cellular basis of learning and memory (Bliss and Collingridge, 1993; Peineau et al., 2007; Komaki and Esteky, 2005). In the hippocampal formation, LTP can be induced by repetitive activation of afferent pathways (Mott et al., 1990; Douglas and Goddard, 1975). LTP can be modulated by several neurotransmitter/neuromodulator systems (Almaguer-Melian et al., 2005). In particular, previous studies clearly demonstrate that the endocannabinoid (eCB) system is critically involved in the physiological mechanisms underlying learning and memory (Varvel and Lichtman, 2005). For example, retrograde eCB signaling modulates LTP induction, as well as glutamatergic and GABAergic transmission (Carlson et al., 2002; Chevaleyre and Castillo, 2003; Isokawa and Alger, 2005).

The eCB system has been implicated in several physiological processes, including pain modulation, appetite regulation, and cognition (Cravatt and Lichtman, 2004; Di Marzo and Matias, 2005; Jacob et al., 2012). Cannabinoids activate two types of receptors, CB_1 and CB_2, with CB_1 receptors mediating the majority of cannabinoid effects in the central nervous system (CNS; Irving et al., 2002). Cannabinoid receptors are highly expressed in the hippocampus. CB_1 receptor stimulation affects the release of a variety of neurotransmitters in the CNS (Schlicker and Kathmann, 2001), including glutamate (Irving et al., 2002; Barzegar et al., 2015) and GABA (Irving et al., 2002; Nazari et al., 2016). CB_1 receptors also participate in multiple synaptic plasticity mechanisms

* Correspondence to: Department of Physiology, School of Medicine, Hamadan University of Medical Sciences, Shahid Fahmideh Street, Postal Code: 65178/518, Hamadan, Iran.
E-mail addresses: alirezakomaki@gmail.com, Komaki@umsha.ac.ir (A. Komaki).
URL: http://umsha.ac.ir (A. Komaki).

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in several brain areas (Földy et al., 2006; Wilson and Nicoll, 2001; Tahmasebi et al., 2016). The eCBs were first reported to be related to short-term plasticity (Wilson and Nicoll, 2001); subsequent studies demonstrated that the eCB system is also involved in long-term synaptic plasticity. Nevertheless, the effects of cannabinoids on synaptic plasticity are controversial (Terranova et al., 1995; de Oliveira Alvares et al., 2006; Lin et al., 2011; Abush and Akirav, 2010).

CNS network functions depend on the balance between excitation and inhibition. GABA is the major inhibitory transmitter in the CNS (Shew et al., 2000). GABA exerts inhibitory effects on neurotransmission and synaptic plasticity through two types of receptors. $GABA_A$ receptors and the more recently discovered $GABA_B$ receptors are distributed throughout the brain (Brucato et al., 1995; Bowery et al., 1987). $GABA_B$ receptors, which are coupled to G proteins, are localized to both pre- and postsynaptic membranes (Safiulina and Cherubini, 2009; Bettler et al., 2004). Activation of presynaptic $GABA_B$ receptors inhibits neurotransmitter release (Poncer et al., 1997), while postsynaptic $GABA_B$ receptor activation causes the opening of potassium channels and thereby induces neuronal hyperpolarization (Safiulina and Cherubini, 2009; Lüscher et al., 1997). Previous studies showing $GABA_B$ receptor-mediated modulation of LTP induction in the dentate gyrus (DG) in vitro suggest a physiological role of $GABA_B$ receptors in learning and memory (Mott et al., 1990; Brucato et al., 1995; Mott and Lewis, 1991). Additionally, endogenous GABA has been shown to regulate LTP induction via activation of $GABA_B$ receptors (Mott et al., 1990).

Although the precise mode of action of cannabimimetic agents on hippocampal networks remains controversial, several studies have suggested that modulation of GABAergic transmission is an important component of their effects (Katona et al., 1999; Paton et al., 1998). Neurochemical (Katona et al., 1999) and electrophysiological studies (Hájos et al., 2000; Hoffman and Lupica, 2000) have revealed that cannabinoids regulate GABA release from inhibitory neurons in the hippocampus (Irving et al., 2002). Electrophysiological and immunocytochemical studies have demonstrated CB_1 receptor expression in the axon terminals of hippocampal GABAergic neurons (Hájos et al., 2000; Ronaghi et al., 2015; Andó et al., 2012) as well as abundant cell-surface CB_1 receptor immunolabeling in the axons of these neurons (Irving et al., 2002, 2000). Activation of CB_1 receptors in GABAergic neurons leads to a decrease in GABA release (Katona and Freund, 2008; Howlett et al., 2002), which results in depolarization-induced suppression of inhibition (Monory et al., 2006) and long-term depression of inhibitory GABAergic synaptic transmission (Azad et al., 2004; Albayram et al., 2011). However, several other lines of evidence have suggested that cannabinoids may also facilitate GABAergic transmission by blocking its reuptake (Varvel and Lichtman, 2005).

Although the eCB and GABAergic systems are known to play important roles in learning, memory, and synaptic plasticity, the interactive effects of these systems on LTP induction in the hippocampal DG in vivo are not clear (Fig. 1). Hence, it is crucial to understand the precise cellular functions of CB_1 and $GABA_B$ receptors in the hippocampus, as well as the role of the eCB system in the modulation of hippocampal GABAergic networks, In this study, we test the hypothesis that the effects of the eCB system on learning, memory and synaptic plasticity result, in part, from its effects on GABAergic synaptic transmission.

2. Results

2.1. Measurement of evoked potentials

We obtained field potential recordings from the granular cell layer of the DG following HFS of the perforant path (PP). Sample traces from each group are illustrated in Fig. 2. Population spike (PS) amplitude was measured from the peak of the first positive deflection of the evoked potential to the peak of the following negative deflection. Field excitatory postsynaptic potential (fEPSP) slope was measured as the slope of the line connecting the start of the first positive deflection of the evoked potential with the peak of the second positive deflection. fEPSPs and PS amplitude were calculated using eTrace data analysis software (ScienceBeam, Tehran, Iran).

2.2. Effects of HFS on PS amplitude and fEPSP slope

Investigation of HFS-induced LTP in the hippocampal DG in vivo revealed a strong enhancement of PS amplitude, which resulted in significant LTP at PP-DG synapses in the control (DMSO + Saline) group ($168.21 \pm 10.85\%$ of pre-HFS baseline; $n = 10$). Further, the mean fEPSP slope following HFS was $119.61 \pm 5.19\%$ of baseline in the control group.

2.3. Effects of CB_1 and $GABA_B$ receptor agonists on fEPSP slope and PS amplitude

HFS applied to the PP-DG area induced LTP in animals treated with AM251, as shown by an increase in fEPSP slope ($138.4 \pm 6.13\%$ of pre-HFS baseline; $n = 10$; Fig. 3). The mean PS amplitude was $231.79 \pm 22.29\%$ of baseline in the AM251 group ($n = 10$; Fig. 4). AM251 administration resulted in significantly greater fEPSP slopes in comparison with the control group ($F[3,32] = 23.85$, $P < 0.001$). PS amplitudes were also significantly higher in AM251-treated animals compared to vehicle-treated controls ($F[3,32] = 15.35$, $P < 0.01$). Baclofen treatment also resulted in significantly higher fEPSP slopes ($184.54 \pm 17\%$; $F[3,32] = 28.49$; $P < 0.001$; $n = 8$; Fig. 3) and PS amplitudes ($342.37 \pm 45.62\%$; $F[3,32] = 19.71$; $P < 0.01$; Fig. 4) in comparison with the control group.

2.4. Effects of CB_1 and $GABA_B$ agonist co-administration on fEPSP slope and PS amplitude

To evaluate the interactive effects of the eCB and GABAergic systems on synaptic plasticity, we investigated the effects of AM251 and baclofen co-administration on LTP. We found that co-administration of AM251 and baclofen resulted in larger fEPSP slopes ($191.96 \pm 29.4\%$; $n = 8$; Fig. 3) and PS amplitudes ($413.28 \pm 67.36\%$; $n = 8$; Fig. 4) than administration of either AM251 (fEPSP slope: $F[3,32] = 23.74$, $P < 0.01$; and PS amplitude: $F[3,32] = 42.56$, $P < 0.001$) or baclofen alone (fEPSP slope: $F[3,32] = 3.92$, $P > 0.05$; and PS amplitude: $F[3,32] = 3.26$, $P > 0.05$). LTP induction in the AM251 + baclofen group was also significantly increased compared to the control group, as shown by increased fEPSP slope ($F[3,32] = 37.64$, $P < 0.001$) and PS amplitude ($F[3,32] = 46.83$, $P < 0.001$).

3. Discussion

In the present study, we investigated the in vivo effects of intrahippocampal infusion of $GABA_B$ and CB_1 receptor agonists on HFS-induced LTP in the rat DG. Activity-dependent synaptic potentiation is expressed as a long-lasting increase in the synaptic component of the evoked response recorded from a large cell

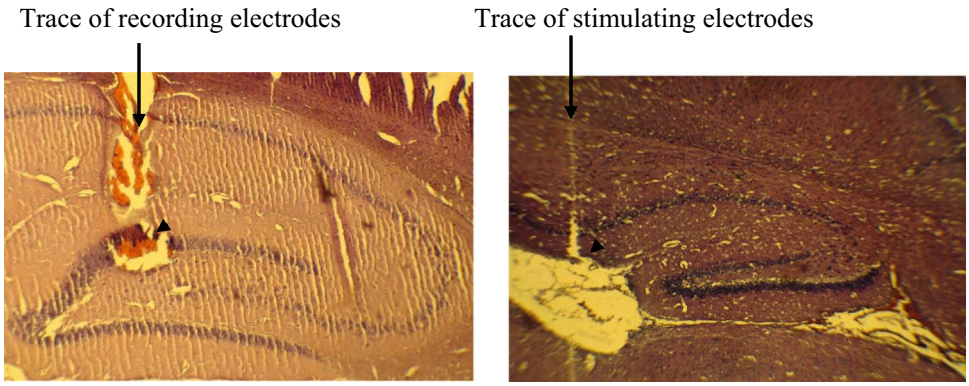

Fig. 1. Representative photomicrograph illustrating locations of recording and stimulating electrodes tips (arrowheads) in a hippocampus sagittal section. Recording and stimulating electrode traces can be seen at the left and right sides, respectively (arrows). Scale bar: 0.5 mm.

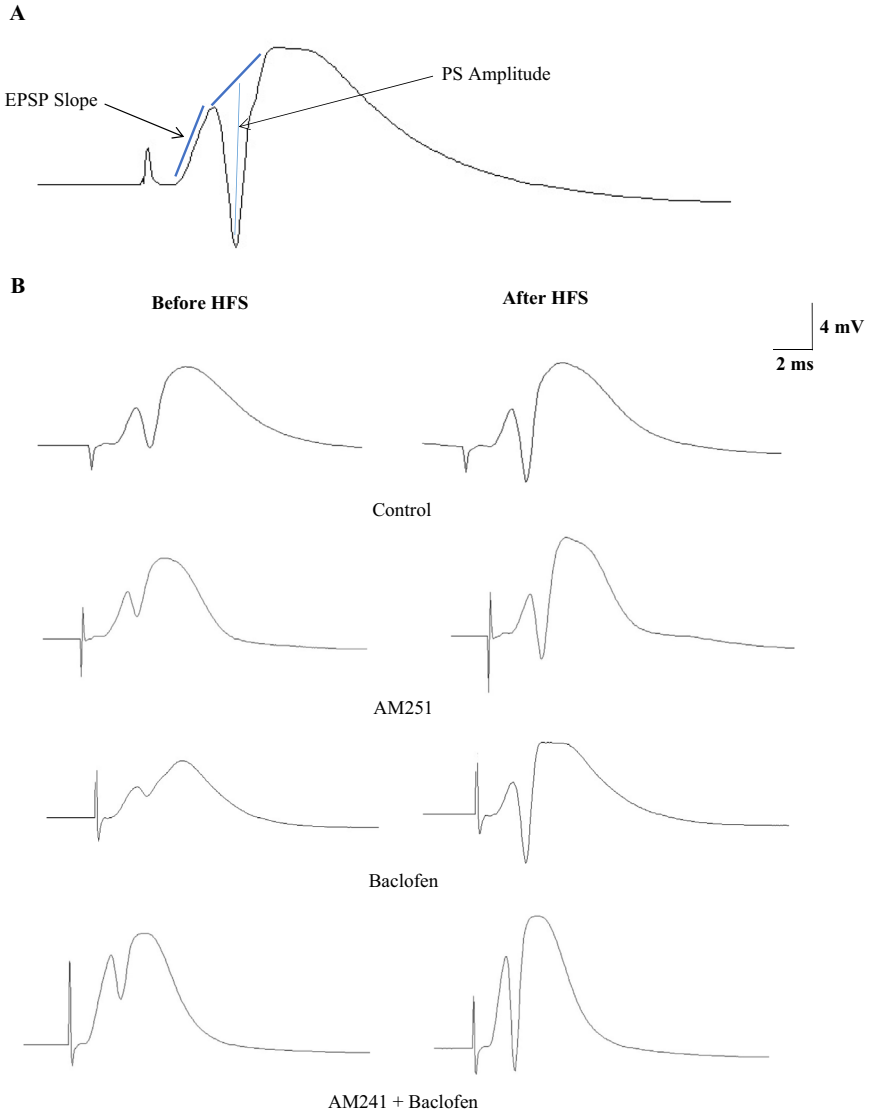

Fig. 2. (A) Field excitatory postsynaptic potential (fEPSP) slope and population spike (PS) amplitude, as measured in a representative sample field potential recording in the hippocampus of a vehicle-treated control animal. Arrows indicate PS and fEPSP slope. (B) Representative sample traces of evoked field potentials recorded in the hippocampal dentate gyrus (DG) prior to and after high-frequency stimulation of the perforant pathway (PP) in all experimental groups.

population; this effect occurs within milliseconds and can persist for hours or days (Di Filippo et al., 2009; Citri and Malenka, 2008). Our results show that baclofen significantly facilitates HFS-induced LTP, as indicated by greater increases in fEPSP slope and PS amplitude. AM51 treatment also significantly increased fEPSP slope and PS amplitude of hippocampal field potentials, which reflect greater increases in LTP. Simultaneous injection of AM251 and baclofen resulted in stronger LTP induction compared to

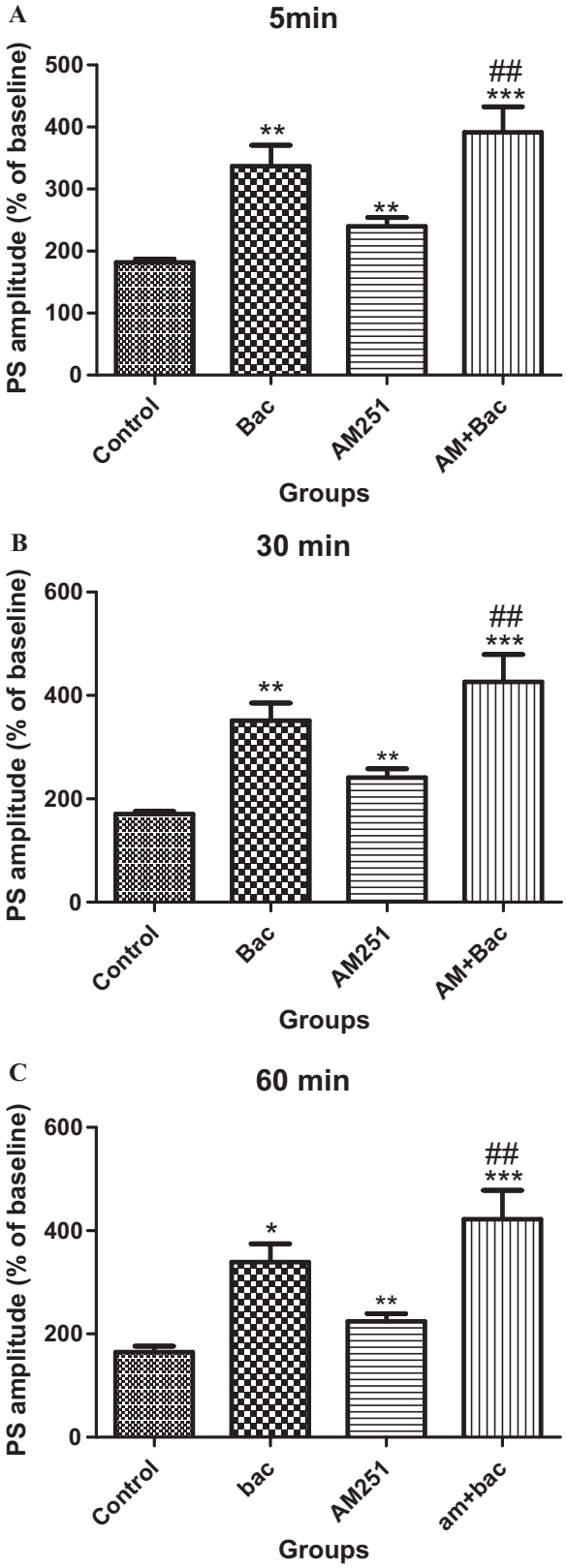

Fig. 3. Effects of AM251 (AM), baclofen (Bac), and AM+Bac on LTP induction in the DG following PP stimulation. Long-term potentiation (LTP) of DG granular cell synapses, as measured by increased PS amplitude, was significantly different between groups. Data are expressed as mean ± SEM % of baseline responses. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle-treated controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ between AM+Bac and AM groups.

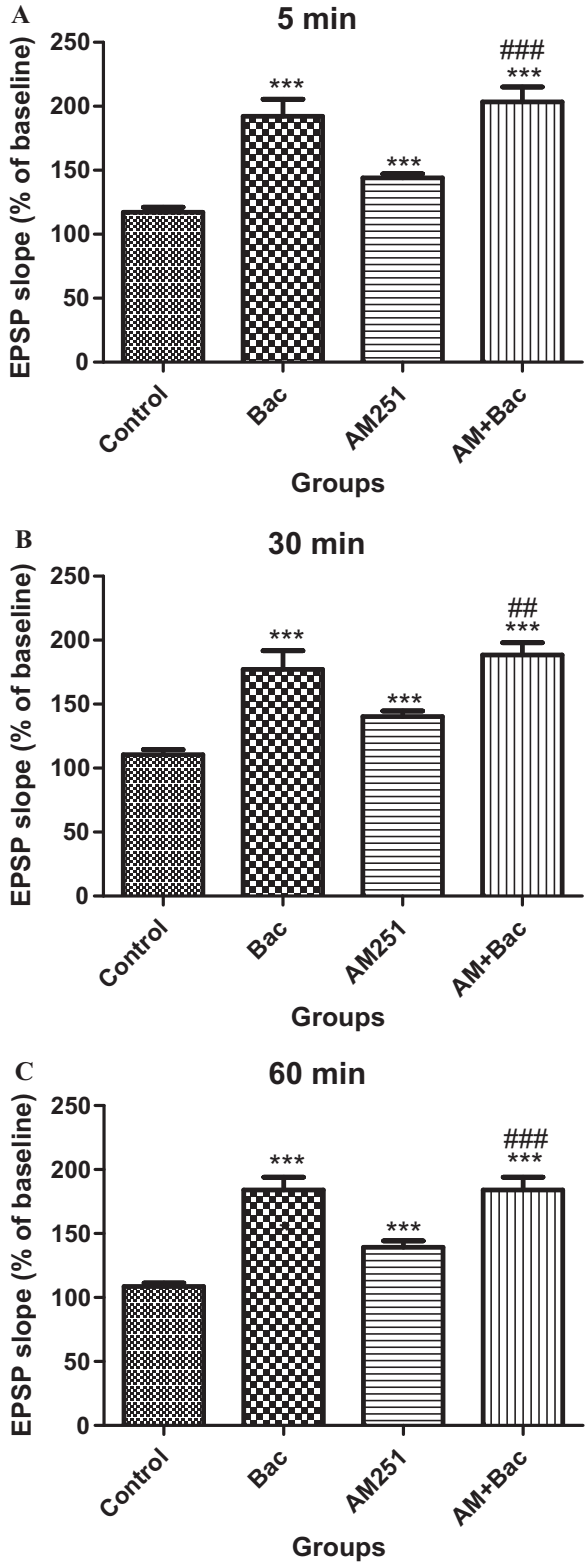

Fig. 4. Effects of AM251 and baclofen on EPSP slope in the DG following 400 Hz tetanic stimulation. Long-term potentiation (LTP) of fEPSP slope in DG granular cell synapses was significantly different between groups. Data are expressed as the mean ± SEM % of baseline responses. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle-treated controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ between AM+Bac and AM groups.

AM251 or baclofen alone.

GABAergic modulation of synaptic plasticity at excitatory synapses can have important functional consequences. By changing the excitatory/inhibitory balance, the GABAergic system can influence excitability and neural circuit function, regulate the inducibility of LTP and long-term depression (LTD) at excitatory synapses, and ultimately contribute to learning and memory (Castillo et al., 2011). Activation of $GABA_A$ receptors increases their Cl^- conductance, which generates the fast inhibitory postsynaptic current (Shew et al., 2000; Eccles et al., 1977). Stimulation of the G-protein coupled $GABA_B$ receptors results in a long inhibitory potassium conductance (Brucato et al., 1995; Bowery, 1993). $GABA_B$ receptors serve various modulatory roles in different areas of the CNS (Mann-Metzer and Yarom, 2002). $GABA_B$ receptors are highly expressed in the prefrontal cortex (Margeta-Mitrovic et al., 1999) and regulate higher cognitive function (Mott and Lewis, 1991; Chalifoux and Carter, 2011). $GABA_B$ receptors can also strongly regulate synaptic activity and plasticity in the hippocampus (Brucato et al., 1995). Presynaptic inhibition is most commonly attributed to $GABA_B$ receptor activation (Mann-Metzer and Yarom, 2002). Multiple studies have demonstrated that stimulation of presynaptic $GABA_B$ receptors on inhibitory neurons decreases GABA release and thereby relieves inhibition (Shew et al., 2000; Brucato et al., 1995; Safiulina and Cherubini, 2009; Davies et al., 1991; Davies and Collingridge, 1993; Olpe et al., 1994). However, little is known about the distribution or effects of postsynaptic $GABA_B$ receptors. $GABA_B$ receptors mediate the slow inhibitory effects of GABA and are crucial to the control of network activity owing to their regulation of neuronal excitability and synaptic transmission (Degro et al., 2015; Kohl and Paulsen, 2010; Palmer et al., 2012; Larkum, 2013). In support of our findings, baclofen has been reported to facilitate the induction of LTP by repetitive stimulation (Mott et al., 1990). Furthermore, in vitro studies have revealed that baclofen can increase the duration of single EPSPs in the DG (Brucato et al., 1995; Mott et al., 1989) and decrease inhibition in the DG by acting on inhibitory neurons (Mott et al., 1990, 1989). Both the pre- and postsynaptic effects of baclofen are mediated by $GABA_B$ receptors that decrease Ca^{2+} currents, likely through their inhibition of many voltage-dependent calcium channel subtypes (Chalifoux and Carter, 2011; Mintz and Bean, 1993; Pfrieger et al., 1994; Lambert and Wilson, 1996).

The effects of cannabinoids on LTP are controversial. Cannabinoid receptor stimulation suppresses presynaptic neurotransmitter release (Misner and Sullivan, 1999). However, cannabinoids can also exert postsynaptic effects on synaptic plasticity (Irving et al., 2002; Hampson et al., 1998; Ong and Mackie, 1999). Previous studies have shown that cannabinoids suppress various forms of synaptic plasticity in the hippocampus, including LTP (Terranova et al., 1995; Misner and Sullivan, 1999; Collins et al., 1994) and LTD (Misner and Sullivan, 1999), via a CB_1 receptor-dependent mechanism (Irving et al., 2002). Studies of CB_1 receptor localization within the CNS have revealed moderate to high densities in several brain areas, including the hippocampus (Katona et al., 1999; Hoffman and Lupica, 2000; Pettit et al., 1998; Tsou et al., 1998). Cannabinoid receptor stimulation suppresses hippocampal LTP by reducing the probability of glutamate release, which in turn prevents postsynaptic neuron depolarization and the subsequent influx of Ca^{2+} through N-methyl-D-aspartate (NMDA) receptors; this effect can be dominated under conditions of enhanced NMDA receptor activation (Irving et al., 2002; Misner and Sullivan, 1999). Similarly, several other studies have shown that acute cannabinoid administration decreases glutamate release, suppresses excitatory synaptic activity, and impairs hippocampal LTP (Ronaghi et al., 2015; Katona et al., 2006). According to our result, administration of CB_1 antagonists in rats and mice results in improved learning and memory in recognition tasks (Jacob et al., 2012; Terranova et al., 1996). Consistent with these findings, injection of AM251 has been reported to enhance LTP induction in rats (Nazari et al., 2016).

Effects of cannabinoids on inhibitory synaptic transmission may be important in controlling the strength of neural activity, which is thought to be critical for learning and memory (Irving et al., 2002; Hájos et al., 2000). Here, we discuss the mechanisms of GABAergic inhibition of synaptic plasticity and the interaction between these mechanisms and the eCB system. High levels of CB_1 receptor immunoreactivity and mRNA are associated with GABAergic interneurons (Irving et al., 2002; Katona et al., 1999; Tsou et al., 1998; Herkenham et al., 1991). CB_1 receptors are expressed by GABAergic interneurons in the hippocampus, and regulation of GABAergic transmission is an important component of their effects (Paton et al., 1998; Ronaghi et al., 2015). In the DG, CB_1 receptors are strongly expressed in cholecystokinin-immunoreactive GABAergic axon terminals, and exogenous application of the synthetic CB_1 receptor agonist WIN55,212-2 reduces inhibitory postsynaptic currents in DG granule cells (Isokawa and Alger, 2005; Hájos et al., 2000). Several previous reports show that regulation of GABAergic system is an important aspect of cannabinoid-mediated effects on LTP (Ronaghi et al., 2015; Collins et al., 1995). In contrast, CB_1 receptor activation is not sufficient to induce LTD, a form of plasticity that can be blocked by hyperpolarizing GABAergic interneurons (Castillo et al., 2011; Heifets et al., 2008). Furthermore, cannabinoid agonists have been shown to inhibit GABA release in several preparations (Varvel and Lichtman, 2005; Wilson and Nicoll, 2001; Katona et al., 1999, 2000; Hoffman and Lupica, 2000). It has also been reported that depolarization-induced postsynaptic Ca^{2+} influx initiates the synthesis and release of an endogenous eCB that inhibits GABA release from presynaptic terminals (Isokawa and Alger, 2005). These behavioral, pharmacological, and physiological data reveal that the efficacy of cannabinoid-mediated control of GABA release is strongly influenced by activity levels in presynaptic interneurons (Földy et al., 2006). Taken together, these results suggest a complex relationship between eCBs and GABAergic effects in the hippocampus.

4. Conclusion

The excitatory neurotransmission in the DG that underlies synaptic plasticity is regulated by $GABA_B$ receptor activity. Our findings support the hypothesis that endogenous GABA released during repetitive firing acts on $GABA_B$ receptors to facilitate LTP induction. Furthermore, our results suggest that CB_1 receptor activation in the hippocampus regulates synaptic transmission onto GABAergic interneurons in the DG. When CB_1 and $GABA_B$ receptor agonists are administered simultaneously, CB_1 agonists can alter GABA release, which in turn enhances LTP. Together, these results suggest that the regulatory effects of cannabinoids on hippocampal LTP occur, in part, through modulation of GABAergic neurotransmission via $GABA_B$ receptors.

5. Materials and methods

5.1. Animals

Thirty-six male Wistar rats (250–300 g) were obtained from the Razi Institute (Tehran, Iran). Animals were housed 3 per cage in a room with controlled temperature (22 ± 2 °C) and a 12–12 h light-dark cycle. Food and water were provided ad libitum. After one week of adaptation, rats were randomly divided into four groups of ten animals each, as follows: (1) Control (DMSO+saline), (2) AM251, (3) baclofen, (4) baclofen+AM251. All research and

animal care procedures were approved by the Veterinary Ethics Committee of this University and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

5.2. Drugs

The CB_1 receptor antagonist 4-methyl-1H-pyrazole-3-carboxamide (AM251; Sigma-Aldrich, Natick, MA, USA) and the non-selective GABA receptor agonist β-(4-chlorophenyl)-γ-aminobutyric acid (baclofen; Sigma) were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in saline solution (0.9% NaCl). The DMSO concentration in both drug solutions was $<10\%$. The same DMSO and saline solution was used as the vehicle. Drugs were unilaterally microinjected into the DG with a Hamilton syringe approximately 20 min prior to high-frequency stimulation (HFS). A volume of 0.5 μl was microinjected over a period of 1 min. Drug concentrations were determined based on previous studies: AM251, 0.1 μg/animal (Nazari et al., 2016; Hakimizadeh and Oryan, 2012; Roohbakhsh et al., 2009); and baclofen, 2 μg/animal (Zarrindast et al., 2002, 2004; Collares and Vinagre, 2010).

5.3. Surgical procedures, electrophysiological recordings, and LTP induction

The procedures for this experiment were similar to those described previously in prior studies using similar methodologies (Nazari et al., 2016; Tahmasebi et al., 2016, 2015; Salehi et al., 2015; Karamian et al., 2015). Briefly, animals were anesthetized with 1.5 g/kg urethane [Ethyl carbamate (Sigma, USA)] (1.5 g/kg with supplemental injections as required) (Karimi et al., 2013; Komaki et al., 2014). Supplementary injections of urethane (0.2–0.5 g/kg) were given when necessary to ensure full anesthesia and placed in a stereotaxic instrument for the in vivo experiments. Small holes were drilled in the skull at the positions of the stimulating and recording electrodes. A heating pad was used to maintain the temperature of the animals at 36.5 ± 0.5 °C. Bipolar electrodes were made from stainless steel wire with teflon insulation and approximately 0.75 mm separated each tip. The stimulating electrode was placed into the perforant pathway (AP= −8.1; ML=4.3; DV=3.2 mm from the skull surface) and the recording electrode into the granule cell layer of the DG (AP= −3.8; ML=2.3; DV =2.7–3.2 mm from the skull surface) respectively according to the atlas of Paxinos and Watson (Paxinos and Watson, 2006). Field potentials corresponded well with previous in vivo studies of the DG (Asadbegi et al., 2016; Nazari et al., 2016; Salehi et al., 2015; Karimi et al., 2015).

When population spike amplitudes were stable, field potentials were generated over a range of stimulus intensities to generate an input-output (I/O) curve. Single 0.1 ms biphasic square wave pulses were delivered through constant current isolation units (A365, World Precision Instruments, Sarasota, FL, USA) at a frequency of 0.1 Hz. The field potential recordings were obtained in the granular cells of the DG following stimulation of the PP. Once a maximal population spike amplitude was established, the current intensity used to generate that response was used throughout the experiment. Stimulation intensity was adjusted to elicit a maximal field population spike (PS) and filed excitatory postsynaptic potentials (fEPSP). The stimulation intensity was adjusted to evoke potentials which comprised 40% of the maximal population spike amplitude, defined by means of an input/output curve. 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. Once a stable baseline was obtained for at least 20 min, LTP was induced using a 400-Hz HFS protocol (10 bursts of 20 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. In order to determine any changes in the synaptic response of DG neurons, both fEPSP and PS were recorded 5, 30 and 60 min after the high frequency stimuli. For each time-point, 10 consecutive evoked responses were averaged at 10 s stimulus interval (Nazari et al., 2016; Tahmasebi et al., 2015; Karamian et al., 2015; Karimi et al., 2015).

5.4. Statistical analysis

Statistical analyses were performed using the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). Data were statistically analyzed using repeated measures ANOVA tests followed by Tukey's test. All results are expressed as the mean ± the standard error of the mean (SEM). A P value of <0.05 was considered statistically significant.

5.5. Histology

The locations of implanted DG and PP electrodes were histologically confirmed in brain sections of animals. The electrophysiological responses are depending on the exact location of electrodes. Recording and stimulating electrodes were located in DG and PP, respectively. At the end of experiment rats were deeply anesthetized with urethane (2.0 g/kg) and perfused through the heart with formol–saline. Brain sections were cut at 50 μm and stained with hematoxylin and eosin for verification of electrode placements (Fig. 1).

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