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Endocannabinoid CB1 receptors are involved in antiepileptogenic effect of low frequency electrical stimulation during perforant path kindling in rats

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ABSTRACT

Introduction: Administration of low-frequency electrical stimulation (LFS) at the kindling site has an anti-epileptogenic effect. In the present study, we investigated the role of cannabinoid receptors type 1 (CB1) in mediating the inhibitory effects of LFS on the development of perforant path kindled seizures.

Methods: For seizure generation, rats were kindled by electrical stimulation of perforant path in semi-rapid kindling manner (12 stimulations per day at 10 min intervals at afterdischarge threshold intensity).To determine the effect of LFS (0.1 ms pulse duration at 1 Hz, 800 pulses) on seizure generation, LFS was applied to the perforant path 5 min after the last kindling stimulation daily. AM281, a CB1 receptor antagonist, was micro-injected into the lateral ventricle immediately after the last kindling stimulation (before LFS application) at the doses of 0.5 and 2 μg/μl during kindling procedure. The expression of cannabinoid receptors in the dentate gyrus was also investigated using immunohistochemistry.

Results: Application of LFS had inhibitory effect on development of kindled seizures (kindling rate). Microinjection of AM281 (0.5 μg/μl) immediately after the last kindling stimulation (before LFS application) reduced the inhibitory effect of LFS on the kindling rate and suppressed the effects of LFS on potentiation (increasing the magnitude) of both population spike amplitude and population excitatory postsynaptic potential slope during kindling acquisition. AM281 pretreatment also prevented the effects of LFS on kindling-induced increase in early and late paired pulse depression. The higher dose of AM281 (2 μg/μl) failed to exert the effects observed with its lower dose (0.5 μg/μl). In addition, there was a decreased CB1 receptors immunostaining in kindled animals compared to control. However, application of LFS following kindling stimulations led to overexpression of CB1 receptors in the dentate gyrus.

Conclusion: Obtained results showed that activation of overexpressed cannabinoid CB1 receptors by endogenous cannabinoids may have a role in mediating the inhibitory effect of LFS on perforant path kindled seizures.

1. Introduction

Deep brain stimulation (DBS) has been proposed for the treatment of pharmaco-resistant epilepsies (Richardson et al., 2003; Velasco et al., 2002). High frequency stimulation (HFS) is traditionally used in brain stimulation therapy; However, low frequency stimulation (LFS; 1–3 Hz) is widely applied because of minimal side effects (Kile et al., 2010). LFS induces inhibitory effects on epileptic activity in animals (Gaito, 1981; Gaito et al., 1980; Goodman et al., 2005; Kile et al., 2010; Ullal et al., 1989; Zhu-Ge et al., 2007) and epileptic patients (Yamamoto et al., 2002). The effectiveness of LFS in treatment of epilepsy has led to renewed research efforts to find its anticonvulsant mechanisms for optimization of the treatment paradigm.

The anticonvulsant mechanism of LFS has not been completely determined; however, mechanisms similar to induction of long-term depression (Fujii et al., 2000; Manahan-Vaughan and Kulla, 2003) and/or depotentiation (Klausnitzer et al., 2004) may be involved. We previously showed that the neurotransmitters and/or neuromodulators which act through Gi protein-coupled receptors, such as adenosine (Mohammad-Zadeh et al., 2009), galanin (Sadegh et al., 2007) and

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dopamine (Rezaei, 2016) have a role in mediating the antiepileptogenic effects of LFS. In this regard, our previous experiment showed that application of LFS during the kindling procedure decreased the expression of regulators of G-protein signaling protein 4 (RGS4) and RGS10 (that reduce the activity of Gi/o). LFS also prevented the kindling-induced decrease of RGS2 protein (which reduces the Gs activity). Therefore, it can be postulated that the Gi/o protein signaling pathways may be involved in antiepileptogenetic effect of LFS (although there was no significant difference in the expression of α-subunit of Gs and Gi/o proteins by themselves) (Namvar et al., 2017).

Compelling evidence has demonstrated the role of endocannabinoid receptors in mediating long term depression (Chevaleyre and Castillo, 2003; Gerdeman et al., 2002; Izumi and Zorumski, 2012) and depotentiation (Izumi and Zorumski, 2016) in different brain regions. Endocannabinoids have also anticonvulsant action both in vitro and in vivo (Jones et al., 2010; Rizzo et al., 2009; Shafaroodi et al., 2004). Therefore, it is possible to consider a role for endocannabinoids in mediating the inhibitory effect of LFS on seizures.

CB1 and CB2 are the two major cannabinoid receptors that belong to the G protein-coupled receptor family and signal through the Go/i family of G proteins. CB1 receptors are found mainly in central nervous system (CNS) and the hippocampus is one of the areas with the highest cannabinoid receptor binding densities in the rat brain (Herkenham et al., 1991). The hippocampus is one of the most seizure-prone structures in the brain (Stringer and Lothman, 1992). Previous observations showed that endocannabinoids, as retrograde messengers, reduce the glutamate or GABA release through presynaptic CB1 receptors (Hajos et al., 2000;Kathmann et al., 1999; Sullivan, 1999). Endocannabinoid signaling mediated through CB1 receptors is important in the control of neuronal excitability and susceptibility of brain to epileptic seizures (Alger, 2004; Wallace et al., 2003). CB2 receptors are expressed in the peripheral-immune system (Elphick and Egertova, 2001; Munro et al., 1993), and exist in CNS at lower amounts compared to CB1 receptors (Gong et al., 2006; Onaivi, 2007). However, there are several reports showing the role of CB2 receptors in controlling of neuronal excitability (Atwood et al., 2012; den Boon et al., 2012; Morgan et al., 2009).

To use the LFS as a new treatment manner for drug-resistant epileptic patients, it is necessary to determine its mechanism of action. Considering the anticonvulsant effects of endocannabinoid receptors and their role in synaptic plasticity, the present study was designed to evaluate the role of endocannabinoid CB1 receptors in mediating the antiepileptogenic protective effects of LFS on perforant path-kindling. For this purpose, we examined whether administration of AM281 before LFS is applied during perforant path kindling procedure can reduce or prevent its effectiveness on kindled seizures.

2. Experimental procedures

2.1. Animals

Sixty-four male Wistar rats (270–300 g at the time of surgery) were kept under standard conditions at 23 ± 2 °C temperature on 12 h dark/light cycle. Animals were housed in individual cages, and food and water were supplied ad libitum. Experiments were performed according to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).We tried to reduce number of rats utilized and to minimize animal pain and suffering. Experiments were performed at the same time of the day to avoid the bias of circadian rhythms.

2.2. Surgical procedure

Under ketamine/xylazine (80/15 mg/kg, respectively) anesthesia, animals were fixed in a stereotaxical apparatus. A bipolar stimulating electrode was implanted into the perforant path (coordinates: 8.1 mm posterior to the bregma; 4.3 mm to the right and 2.5–3 mm below dura). A monopolar recording electrode was implanted into the dentate gyrus (coordinates: 3.8 mm posterior to the bregma; 2.3 mm to the right and 2.7–3.4 mm below dura) of the right hemisphere. The depth of the recording and stimulating electrodes were determined so that following stimulation of perforant path, the maximum population spike (PS) amplitude of the evoked response can be achieved in the dentate gyrus. Electrodes (stainless steel, Teflon coated, 127 μm in diameter, A.M. Systems, Inc., USA) were insulated, except for 0.5 mm at the tips. Before electrodes implantation, two stainless steel screws were positioned above the skull as reference and ground electrodes, and two additional screws were used as anchors. A 23-gauge guide cannula was implanted in the right lateral ventricle (coordinates: 0.9 mm posterior to the bregma; 1.5 mm to the right and 2.5–3 mm below dura). All electrodes were connected to pins of a lightweight multi-channel miniature socket as a head stage. The socket and cannula were fixed to the skull by dental acrylic. After post-operative recovery for at least 10 days, electrophysiological experiments were conducted as described elsewhere (Mohammad-Zadeh et al., 2007).

2.3. Stimulation and recording

Following recovery, the rat was transferred to a recording box (30 × 30 × 30 cm). The rat's socket was connected to a flexible, shielded cable while the rat was allowed to move freely in the recording box. All evoked responses were amplified and digitized (at 10 kHz) by a PC-based electromodule system (Electromodule R12, Science Beam Co., Iran) with custom-designed software, and were continuously monitored and stored on disk.

2.4. Input-output curves

To determine the input-output curve, single 0.1 ms monophasic square wave pulses were applied to the perforant path while evoked field potentials were monitored in the dentate gyrus. The pulses were delivered through a PC-based electromodule system at varying intensities (100–1000 μA) every 10 s. Input-output curves were generated by calculating PS amplitude (the average of 6 evoked responses) at different stimulus intensities. The intensity that produced 50% of maximum PS amplitude was considered a test pulse and used in the subsequent experiments. The measured test pulses were in the range of 100–800 μA in different animals.

2.5. Field potential recording

Obtained test pulses (100–800 μA) were set for the field potential recordings. Field potentials were recorded for 20 min when the animals were stimulated at the intensity equal to their test pulse and the frequency of 0.1 Hz. Field potential recordings were performed on day 1 (before kindling stimulations), and days 4 and 7 during kindling stimulations. The responses of 120 sweeps recorded on day 1 were averaged and considered as 100%. The responses on days 4 and 7 were normalized accordingly. Both PS amplitude and population excitatory postsynaptic potential (pEPSP) slopes were calculated and twelve sweeps were averaged for each time point.

2.6. Paired pulse tests

During field potential recordings, paired pulse tests were run on the days 1, 4, and 7 at the intensity of test pulses (100–800 μA). Six sweeps were averaged at each of 8 inter-pulse intervals (20, 30, 50, 70, 100, 300, 500, and 1000 ms). These inter-pulse intervals were used randomly, and pulse pairs were separated by 10 s (0.1 Hz). Calculation of the percent ratio of the second PS (test) to the first (conditioning) was considered as the paired-pulse index, which is a sign of changes in inhibitory circuits during epileptogenesis and can be used for determining short term plasticity.