Brain Stimulation 6 (2013) 515–523

Contents lists available at SciVerse ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Electrical Low Frequency Stimulation of the Kindling Site Preserves the Electrophysiological Properties of the Rat Hippocampal CA1 Pyramidal Neurons From the Destructive Effects of Amygdala Kindling: The Basis for a Possible Promising Epilepsy Therapy

Zohreh Ghotbedin [a], Mahyar Janahmadi [b,c,*], Javad Mirnajafi-Zadeh [a], Gila Behzadi [b,c], Saeed Semnanian [a]

[a] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Neuroscience Research Centre, Medical School, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran
[c] Department of Physiology, Medical School, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran

ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form
29 September 2012
Accepted 7 November 2012
Available online 24 November 2012

Keywords:
Low frequency stimulation
Amygdala kindling
Firing properties
Antiepileptic
CA1 pyramidal neurons

ABSTRACT

Background: Deep brain stimulation (DBS) has emerged as a potential therapeutic strategy in the treatment of neurological disorders including epilepsy. However, the cellular mechanism responsible for the effects of DBS remains largely undefined. Therefore, using electrophysiological approach, we aimed to determine the antiepileptic effects and restorative potential of low frequency stimulation (LFS) on amygdala kindling-induced changes in electrophysiological properties of rat hippocampal CA1 pyramidal neurons.

Methods: Animals were kindled by electrical stimulation of amygdala in a rapid kindling manner (12 times per day). In one group of animals, immediately after termination of daily 12 rapid kindling stimulations, the kindling site was subjected to 4 packages of LFS at intervals of 5 min (each package contained 200 monophasic square-wave pulses, 0.1 ms pulse duration at 1 Hz). Whole cell patch clamp recording under current clamp conditions was performed on visually identified pyramidal neurons in hippocampal slice preparations obtained from amygdala-kindled rats and the rats receiving LFS.

Results: Kindling of the right basolateral amygdala profoundly affected spontaneous firing behavior and repetitive discharge characteristics of pyramidal neuronal electrophysiological properties. Application of LFS at the kindling site almost completely prevented the development of epilepsy and the disruptive effects of kindling on neuronal electrical activity through restoration of the normal electrophysiological characteristics.

Conclusions: The results of this study implied that application of LFS during kindling acquisition prevents the kindling induced changes in functional electrical properties of CA1 pyramidal neurons, suggesting that this action may be involved in the antiepileptogenic mechanism of LFS.

Introduction

Epilepsy that affects 1–2% of the world population [1] is a devastating chronic neurological disorder characterized by recurrently and repeatedly occurring seizures. There is no definite radical therapy for epilepsy and, on the other hand, antiepileptic drugs (AEDs) are unable to provide a persistent cure; that is, AEDs are merely anti-seizure, but do not prevent epileptogenesis [2]. Temporal lobe epilepsy (TLE) is the epilepsy syndrome most resistant to antiepileptic drugs [3] and involves recurrent seizures arising from the anterior medial temporal lobes of the brain, such as the hippocampus and amygdale [4]. Selective amygdalaohippocampectomy can be a good therapy option for treating drug resistant patients with TLE. However, for many patients with refractory epilepsy, finding an identifiable epileptogenic focus is not easy [5–7]. Therefore, alternative therapies are needed. The use of deep brain stimulation, which is currently being studied both in experimental animals and in patients with epilepsy [8–12], could be a promising approach for treating uncontrolled epilepsy. Compared to epilepsy surgery, electrical stimulation including LFS is safe, reversible (switching on and off), and with least damage to the surrounding neuronal structures [13]. Important stimulation parameters including frequency, pulse width and amplitude also

This work was supported by a grant (No. 90002200) from Iran National Science Foundation (INSF).

* Corresponding author. Neuroscience Research Centre, Medical School, Shahid Beheshti University of Medical Sciences, Evin, P.O. Box: 19615-1178, Tehran, Iran. Tel./fax: +98 21 22439971.
E-mail addresses: Janahmadi@sbmu.ac.ir or mjanahmadi@yahoo.com (M. Janahmadi).

1935-861X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.brs.2012.11.001

can be adjusted [14]. Gaito and colleagues [15] reported that LFS (1–3 Hz) produces strong and long-lasting inhibition of kindling-induced epileptic activity. It has also been reported to successfully suppress seizures [16–18], including amygdaloid-kindled seizures [19]. Studies on brain slices also revealed that LFS can block epileptiform activity [20,21]. Therefore, LFS could be considered as a good alternative option in DBS treatments for untreatable epilepsy compared to high frequency stimulation (HFS) because of its lower risk of complications [22–24].

Despite extensive investigation demonstrating the potential beneficial effects of LFS on epilepsy and seizure disorders, our knowledge of the cellular consequences that underlie these effects are still incomplete and there are few studies in which the possible mechanism of the therapeutic action of DBS has been attributed to the accumulation of adenosine [25], secretion of brain-derived neurotrophic factor [26], elevation of cAMP [16], modification of receptor binding [27] or alteration of neuronal activity [28,29]. In the present study, however, emphasis has been focused on determining the amygdala-kindling induced alterations in the electrophysiological properties of CA1 pyramidal cells and to elucidate how daily LFS immediately after kindling can prevent these changes from occurring.

Materials and methods

Animals and surgery

A total of 40 young adult male Wistar rats (4–6 weeks old) were maintained in individual cages under standard laboratory conditions (ambient temperature 23–25 °C, 12 h light/dark alternate cycle with lights on between 7:00 AM and 7:00 PM, food and water ad libitum). All experiments were designed to minimize the number of rats used and carried out via a protocol approved by Shahid Beheshti Medical Sciences University Animal Ethic Committee and was in complete compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgical procedure and induction of amygdala kindling

Twenty rats were anesthetized with ketamine/zylazine mixture (100/10 mg/kg, i.p.) and then a tripolar twisted electrodes (a bipolar stimulating and a monopolar recording) made of three 127 μm in diameter stainless steel, Teflon coated wires insulated except at their tips (A.M. system, Inc., USA), was stereotaxically implanted into the basolateral amygdale (BLA) of the right hemisphere using coordinates of Paxinos and Watson atlas [30]: anteroposterior: −2.5 mm; lateral: 4.8 mm; vertical: 7.2 ± 0.2 mm below the skull and incisor bar was set at 2.9–3.1 mm below interaural line. Electrodes were connected to plugs, and the electrode assembly and anchor screws were fixed in place with acrylic cement applied to the surface of the skull. The electrode location was histologically confirmed in animals at the end of the experiments. To do this, rats were deeply anesthetized and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) prior to neutral red staining to verify electrode placements (Fig. 1).

Induction of rapid kindling

In the present study rapid kindling paradigm was used. The rapid kindling has been reported to be similar to the conventional kindling except that in conventional procedure stimulations are daily delivered until stage 5 seizures are elicited on 3 consecutive days, whereas in rapid kindling procedure stimulations are applied until animal exhibits consistent stage 5 seizures [31], which is considered to be the endpoint of kindling. It has been reported that

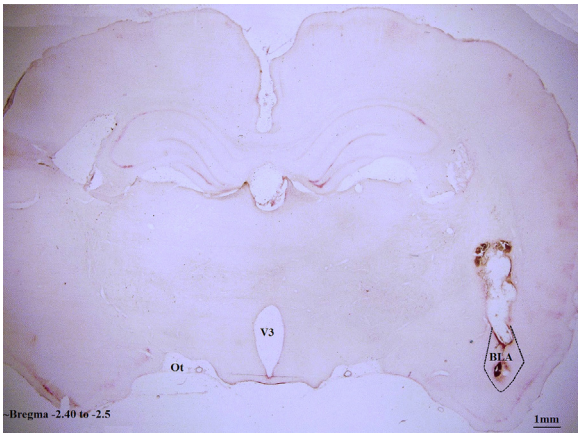

Figure 1. Histological verification of electrodes position in amygdale. Representative photomicrograph of neutral red stained section showing the position of the stimulating and recording electrodes in the right basolateral amygdala (BLA). V3 = third ventricle; Ot = optic tract.

rapid kindling, as a model of epileptogenesis, allows accelerated evaluation of experimental manipulation during the progression of epilepsy induction [31–33], therefore, it can be used as a model of compressed epileptogenesis as an alternative kindling paradigms to the conventional models [33]. After ten days of postsurgical recovery, in kindled group of rats ($n > 10$) rapid electrical kindling was performed by amygdala stimulations using 3 s, 50 Hz monophasic square-wave pulses of 1 ms, which were applied 12 times per day with 5 min intervals. Stimulation was performed using an electrical stimulator and a stimulus isolator (SS-202J, Nihon Kohden, Japan). The stimulus intensity initially began at 30 μA and then increased in 10 μA steps until at least 8 s of afterdischarges (AD) was elicited. The minimal intensity necessary to produce AD was designated as the AD threshold for that animal, and was used for daily stimulation.

Electroencephalograms (EEGs) at the amygdala were recorded and the seizure severity was rated according to Racine's scale [34]: Stage 0, rats showed no convulsion; stage 1, rats showed facial automatism; stage 2, head nodding, stage 3, unilateral forelimb clonus, stage 4, bilateral forelimb clonus; stage 5, rearing, falling and generalized convulsions. In KLFS group, immediately after termination of daily 12 rapid kindling stimulations, the kindling site was subjected to 4 packages of LFS at intervals of 5 min (each package contained 200 monophasic square-wave pulses, 0.1 ms pulse duration at 1 Hz). At the beginning of the experiments, there was no significant difference between the mean AD thresholds (82.22 ± 10.53 μA in kindled rats versus 83.75 ± 8.99 μA in KLFS animals). In kindled rats the slices preparation was made 24 h after observing the first stage 5 seizure (i.e. when the animals are considered as kindled animal). The number of stimulation days in KLFS group was considered equal to the mean stimulations days required for kindled animals to achieve a stage 5 seizure.

In addition to the kindled and KLFS groups ($n = 10$ rat in each group), two aged matched groups, including electrode-implanted but not kindled rats (as sham control, $n = 10$), and naive control rats (as non implanted, $n = 10$) were also used. As no significant differences in electrophysiological results between sham and naïve groups were found, the data of two groups were pooled.

Hippocampal slice preparation and whole cell patch clamp recording

Electrophysiological recording was performed as previously described [35]. Briefly, young adult rats were anaesthetized with ether, euthanized by decapitation, and the brains were quickly