Epilepsy Research (2012) 99, 69—77

journal homepage: www.elsevier.com/locate/epilepsyres

Effect of low-frequency electrical stimulation parameters on its anticonvulsant action during rapid perforant path kindling in rat

Marzieh Shahpari[a], **Javad Mirnajafi-Zadeh**[a,*], **Seyed Mohammad P. Firoozabadi**[b], **Ali Yadollahpour**[b]

[a] *Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran*
[b] *Department of Medial Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran*

Received 31 May 2011; received in revised form 13 October 2011; accepted 15 October 2011
Available online 8 November 2011

KEYWORDS
Epilepsy;
Low frequency stimulation;
Kindling;
Dentate gyrus

Summary Low frequency stimulation (LFS) may be considered as a new potential therapy for drug-resistant epilepsy. However, the relation between LFS parameters and its anticonvulsant effects is not completely determined. In this study, the effect of some LFS parameters on its anticonvulsant action was investigated in rats. In all animals, stimulating and recording electrodes were implanted into the perforant path and dentate gyrus, respectively. In one group of animals, kindling stimulations were applied until rats achieved a fully kindled state. In other groups, different patterns of LFS were applied at the end of kindling stimulations during twenty consecutive days. In the first experiment the effect of LFS pulse numbers was investigated on its anticonvulsant action. Animals were divided randomly into three groups and 1, 4, and 8 packages of LFS (each pack contains 200 pulses, 0.1 ms pulse duration at 1 Hz) were applied five minutes after termination of kindling stimulations. Obtained results showed that 4 packages of LFS had the strongest anticonvulsant effects. Therefore, this pattern (4 packages) was used in the next experiment. In the second experiment, 4 packages of LFS were applied at intervals of 30 s and 30 min after termination of kindling stimulations. The strongest anticonvulsant effect was observed in the group received LFS at the interval of 30 s. Therefore, this pattern was selected for the third experiment. In the third experiment the effect of LFS at frequencies of 0.25 Hz and 5 Hz was investigated. The group of animals which received LFS at the frequency of 0.25 Hz showed somehow stronger anticonvulsant effect. The results indicate that different parameters of LFS have important role in induction of LFS anticonvulsant effects. Regarding this

* Corresponding author at: Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, PO Box 14115-331, Tehran, Islamic Republic of Iran. Tel.: +98 21 82883865; fax: +98 21 82884555.
E-mail address: mirnajaf@modares.ac.ir (J. Mirnajafi-Zadeh).

0920-1211/$ — see front matter
doi:10.1016/j.eplepsyres.2011.10.023

view, it seems that the slower LFS frequency and the shorter interval between LFS and kindling stimulations, the stronger anticonvulsant effect will be observed. But there is no direct relation between number of pulses and the magnitude of anticonvulsant effect of LFS.

Introduction

Epilepsy is one of the most common neurological disorders. Unfortunately, about 20% of epileptic patients are resistant to drug therapy (Schiller and Bankirer, 2007). Surgical treatment may also be useful in the patients with focal epilepsies; but, it has a lot of irreversible side effects and may lead to neurological or cognitive impairments (Wiebe et al., 2001). Thus, there is a need to find new treatment strategies for these patients. Recently, deep brain stimulation has emerged as a new treatment for drug-resistant epilepsies (Li and Mogul, 2007). Application of deep brain stimulation with high frequency stimulus (HFS, >5 Hz) in different brain regions such as the centromedian thalamus (Velasco et al., 2001), anterior thalamus (Kerrigan et al., 2004), subthalamic nuclei (Benabid et al., 2002) and substantia nigra pars reticulata (Feddersen et al., 2007) has antiepileptic effects. However, repeated HFS may aggravate seizure (Feddersen et al., 2007), cause tissue damage and inactivate or inhibit the stimulated sites (Burbaud et al., 2002; Wu et al., 2008).

In 1980s, in a series of classical experiments, Gaito showed that application of low frequency stimulation (LFS, <1—3 Hz) could result in long lasting inhibition of epileptic activity induced by kindling (Gaito, 1981a,b; Gaito et al., 1980). At the same time it was also reported that application of LFS pulses with special duration and intensity could produce kindled seizures (Cain and Corcoran, 1981; Corcoran and Cain, 1980). However, the antiepileptic effects of LFS have been shown in many studies (Ang et al., 2006; Ghorbani et al., 2007; Velisek et al., 2002; Weiss et al., 1995; Wu et al., 2008; Yamamoto et al., 2002; Zhu-Ge et al., 2007). These effects of LFS mainly depend on two factors: the stimulation targets and the stimulus parameters. Seizure-prone areas of the brain may be suitable targets for LFS application. Among these areas, the dentate gyrus plays an important role in temporal lobe epilepsy and is one of the most sensitive areas for induction of kindled seizures (Ang et al., 2006; Morimoto et al., 2004). Thus, it may have a role in the anticonvulsant actions of LFS. Our previous studies also showed the effectiveness of LFS applied in the dentate gyrus on kindled seizures (Jahanshahi et al., 2009; Mohammad-Zadeh et al., 2007, 2009; Sadegh et al., 2007).

Among different LFS parameters, the frequency, time of application and the number of its pulses seem to be very effective in its anticonvulsant effects. These parameters are also important in induction of depotentiation and long-term depression (LTD) by LFS (Kemp et al., 2000; Huang et al., 1999; Martin, 1998; Straube and Frey, 2003). According to previous studies, a wide variety of LFS patterns can suppress seizures (Table 1). However, it is not possible to determine the relation between LFS parameters and the severity of its anticonvulsant effects by comparing these studies. We previously showed that some LFS parameters, including pulse duration, intensity and train duration may affect its anticonvulsant effects in piriform cortex kindling (Ghorbani et al., 2007). Considering the importance of frequency, time of application and pulse numbers on LFS action, in this study the effect of changes in these parameters on LFS anticonvulsant effects were investigated during rapid perforant path kindling.

Materials and methods

Animals

Male Wistar rats (280—300 g at the time of surgery) obtained from the Pasteur Institute of Iran (Tehran, IR Iran) were maintained in a colony room kept at a constant temperature with an artificial 12-h light/dark schedule. The lights were turned on at 7:00 AM. Animals were housed in individual cages with woodchip bedding and permitted free access to standard food and water. Efforts were made to reduce animal suffering and to minimize the number of used animals. All studies were performed in accordance with the ethical guidelines set by the ''Ethical Committee of School of Medical Sciences, Tarbiat Modares University,'' which completely coincides with the ''NIH Guide for the Care and Use of Laboratory Animals.'' All experiments were done at the same time (8:00 AM to 2:00 PM).

Surgical procedure

Surgical procedure was done by using a strereotaxic instrument with the incisor bar set 3.3 mm below the interaural line as described previously (Mohammad-Zadeh et al., 2007). Animals were anesthetized by sodium pentobarbital (50 mg/kg, i.p.). A bipolar stimulating electrode, consisted of two twisted electrodes with a tip distance of 0.5 mm, was implanted in the perforant path (coordinates: *A*, 6.9 mm; *L*, 4.1 mm; and, *V*, 2.0—2.5 mm below dura) of the right hemisphere. A monopolar recording electrode was also implanted in the dentate gyrus (coordinates: *A*, 2.8 mm; *L*, 1.8 mm; and, *V*, 2.5—3.0 mm below dura) of the same hemisphere (Paxinos and Watson, 1986). Two other electrodes connected to stainless steel screws were positioned in the skull above the frontal and occipital cortices as reference and ground electrodes. Electrodes were stainless steel, Teflon coated, 127 μm in diameter, and insulated except at their tips (A-M Systems, Inc., WA, U.S.A.). The other end of each electrode was connected to a pin of a lightweight multichannel miniature socket.

The depth of the recording and stimulating electrodes was adjusted to maximize the population spike amplitude in the dentate gyrus in response to the perforant path stimulation and to confirm the location of the electrodes. For population spike recording, single 0.1 ms monophasic square wave pulses were delivered through Nihon Kohden (Japan, Tokyo) stimulator and Nihon Kohden SS-202J constant-current stimulus isolation unit every 10 s. These pulses were applied to the perforant pathway at different intensities (100—800 μA) while the evoked field potentials were monitored in the dentate gyrus. After recording the maximum population spike amplitude, all electrodes were fixed in the miniature socket (as a