Low- and high-frequency electric cortical stimulation suppress the ferric chloride-induced seizures in rats

Qing-He Yao¹, Hua Zhang¹, Hai-Wei Wang, Xiao-Rong Jing, Heng Guo, Guo-Dong Gao*

Department of Neurosurgery, Tangdu Hospital, Fourth Military Medical University, Xin-shi Road, Shaanxi Province, Xi’an 710038, China

Received 19 July 2007; received in revised form 27 August 2007; accepted 22 October 2007

Abstract

The clinic treatment of epilepsy with epileptic foci overlapped with eloquent cortex is not satisfactory. In this study we investigated the direct effects of low- and high-frequency electric cortical stimulation (ECS) on ferric chloride-induced seizures in the experimental rats. Results showed that spontaneous seizures were observed in all rats during the EEG recording after the intracortical injection of ferric chloride solution into left sensorimotor cortex. One-hertz or 100-Hz ECS with 0.3 ms duration and 0.1 mA amplitude square pulses in 1 h on the cortical lesioned area significantly decreased the number of seizures compared with that of the non-stimulation control group. The mean duration time of seizures in 1-Hz or 100-Hz groups was apparently shorter than that in the control group. In brief, this study showed that both low- and high-frequency ECS suppressed the seizures induced by ferric chloride in rats, indicating their potential treatment effects on epilepsy in clinic.

Keywords: Electric cortical stimulation; Epilepsy; Ferric chloride; Rat

For epilepsy surgery, by preoperative evaluation, the epileptic focus in many patients could be localized and a good outcome is generally followed by a resection of the epileptogenic cortex. But when the epileptogenic cortex is overlapped with eloquent cortex, risk of resection may not be acceptable. In such situation, multiple subpial transaction (MST) may be an alternative surgery. However, MST cannot make seizure completely disappeared. In addition, although the seizure is inhibited significantly, there are many risks related to MST, such as hemorrhage or nerve injury.

Electric stimulation is an encouraging strategy for the treatment of intractable epilepsy. Previous studies have shown that electric stimulation may be useful for epilepsy and other conditions [1,11]. Some researchers found that 1-Hz electric stimulation, applied after kindling stimulation of the amygdala, inhibited the development and expression of amygdala-kindled seizures [15]. Recent studies in brain slices have shown that electric stimulation, especially the low-frequency electric stimulation, can produce an inhibitory effect on seizures [2,4,9,12,17]. However, the effect of electric cortical stimulation (ECS) directly on epileptic focus is not clear. In this study we injected intracortically ferric chloride into left sensorimotor cortex in rats, and investigated the possible effects of low- and high-frequency ECS on ferric chloride-induced seizures.

Experiments were performed on adult male Sprague-Dawley rats (300–350 g). Animals were provided by Laboratory Animal Center of the Fourth Military Medical University (FMMU) and use of the animals was reviewed and approved by the FMMU Animal Care and Use Committee. Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The rat was placed in a stereotaxic frame and a midline incision was made along the scalp. The skull was exposed and a burr hole was made in the location over the left sensorimotor cortex, 2 mm posterior and 2 mm lateral to Bregma. Ferric chloride solution (100 mM, 5 μl; dissolved in saline, pH 2.2) was injected 2.5 mm below skull at a rate of 1 μl/min through a microinjection syringe. The needle remained for 1 min after injection. Then six stainless steel screw electrodes were placed epidurally 2 mm lateral to sagittal suture on both sides: 2 mm anterior (named as Fp1 and Fp2, respectively), 2 mm posterior (named as F3 and F4, respectively) and 6 mm posterior (named as C3 and C4, respectively) to the Bregma. The electrodes named as odd Arab number were planted over the left hemisphere. The screw electrodes and the connected wire were fixed and insulated with dental resin. Eighteen rats were randomly divided into three groups, with six...
rats in each group. In one group named SG1 rats were treated with low-frequency (1 Hz) square pulse ECS (duration of 0.3 ms and amplitude of 0.1 mA, which not easily induced kindling, cathode attached to the F3 electrode and anode attached to the Fp1 and C3 electrode) through epidural electrodes. The starting of ECS was at completely recovering time point from anesthesia and the stimulation was applied for 1 h. In another group named SG100 rats were treated with high-frequency (100 Hz) square pulse ECS just identical to that of SG1. The rats treated with the sham-stimulation were served as control group (CG).

To ensure rats awaking during EEG recording and to avoid the immediate effects of the ECS, we performed the EEG recording starting at 1 h after ECS and continuously monitored for 6 h. We detected the beginning of the seizure when the high frequency synchronous spike activity and/or spike-slow wave appeared and detected the end of the seizure when the high frequency synchronous spike activity and/or spike-slow wave stopped and postictal depression appeared in EEG. The number of seizures and the duration of each seizure were recorded during the 6 h EEG recording. SPSS11.0 (Statistical Program for the Social Sciences, a statistical software of SPSS Inc., USA) was used in data analysis. One-way ANOVA with Dunnett’s t-test was used for the statistical significance and \( P < 0.05 \) was considered significant.

No rat died during the operation and EEG recording. Spontaneous seizures were observed in all rats during the EEG recording. A typical seizure of EEG showed high frequency spike activity and/or spike-slow wave appeared and detected the end of the seizure when the high frequency synchronous spike activity and/or spike-slow wave stopped and postictal depression appeared in EEG. The number of seizures and the duration of each seizure were recorded during the 6 h EEG recording. SPSS11.0 (Statistical Program for the Social Sciences, a statistical software of SPSS Inc., USA) was used in data analysis. One-way ANOVA with Dunnett’s t-test was used for the statistical significance and \( P < 0.05 \) was considered significant.

No rat died during the operation and EEG recording. Spontaneous seizures were observed in all rats during the EEG recording. A typical seizure of EEG showed high frequency spike activity and/or spike-slow wave, following postictal suppression pattern (Fig. 1). Many seizures started from one channel and generalized to the other ones, whereas it was difficult to distinguish which channel started first in most seizures.

One-hertz or 100-Hz ECS on the cortical lesioned area significantly decreased the number of seizures (6.67 ± 5.65, \( P < 0.01 \) and 8.33 ± 5.38, \( P < 0.05 \), in 6 h EEG recording, respectivley) compared with that of the control group (18.33 ± 6.93). The mean reduction rate was 63.61 ± 15.86% in SG1 and 54.56 ± 13.97% in SG100, respectively (Fig. 2). When we calculated the number of seizures in each hour after ECS, we found that downtrend was observed in SG1 and SG100 and transient upgrade followed downtrend was observed in CG (Fig. 3). We also measured the duration of seizures in three groups. After the treatment of 1-Hz or 100-Hz ECS on the lesioned area, the duration of seizures was also decreased significantly, with 37.38 ± 15.53 s (\( P < 0.05 \)) in SG1 and 39.60 ± 11.82 s (\( P < 0.05 \)) in SG100, compared with 61.43 ± 16.61 s in CG (Fig. 4).

Electric stimulation has been used in the treatment of epilepsy for a long time [1]. There are many targets which can be selected to stimulate, such as cerebellum, vagus nerve, thalamus, fornix.

Fig. 1. One example of seizure EEG recording at the time point that 5 h after the intracortical injection of ferric chloride in a control rat. Rat was injected with 5 µl of ferric chloride solution (100 mM) into the left sensorimotor cortex. The recording was from six epidural stainless steel screw electrodes described in experimental procedure. Ictal pattern occurs in all leads with postictal depression.
and hippocampus. But the electric stimulation of targets above is non-specificity to epileptogenic cortex. The ECS directly on epileptogenic cortex maybe an effective method to treat epilepsy, especially focal onset seizure raised from eloquent cortex. The present study demonstrated that 1-Hz or 100-Hz square pulse ECS with duration of 0.3 ms, amplitude of 0.1 mA, directly on the lesioned area can significantly decrease the number and duration of seizures induced by ferric chloride in rats.

Previous study has shown that exogenous electric fields can suppress epileptiform discharge in brain slices [2]. Some studies reported that 1-Hz electric stimulation, applied after kindling stimulation of the amygdala, inhibited the development and expression of amygdala-kindled seizures in rats [14,15]. Although the suppressive effects were demonstrated in these studies, the amygdala-kindled rats were quite different from the neocortical focal epilepsy in the clinic. In present study, we made the epileptic model in rats by intracortical injection of ferric chloride into sensorimotor cortex. The mechanism of this model is similar to posttraumatic epilepsy and it was reported that focal electro seizure discharges were induced after injection of ferrous or ferric chloride solution on rat cerebral cortex [16]. The transient upgrade followed downtrend of the number of seizures suggested that the response of intracortical injection of ferric chloride was severe and gradually improved in rats. The 1-Hz or 100-Hz ECS reduction of number and duration of seizures by direct stimulation of the lesioned area on sensorimotor cortex implied the suppressive effect on focal epilepsy. Our results had been in concordance with the previous clinic studies, the suppression of epileptiform discharges by low frequency of ECS had been found on epileptic focus in individual patient [9,12,17]. Low-frequency (1-Hz) electric stimulation could induce long-term depression (LTD) in rat hippocampal slices [2], motor cortex slices [7] and human temporal cortex slices [5]. LTD is thought to be one of the main mechanisms underlying the inhibitory effects of epileptiform activity by modifications of synaptic efficacy. Another possible mechanism is involvement of GABA-benzodiazepine and endogenous opioid systems in nerve system [10]. We think that these possible mechanisms maybe work together to suppress the seizures.

In present study, we demonstrated that both 1-Hz and 100-Hz ECS can suppress seizures in ferric chloride-induced epileptic rats. This result was especially interesting. High-frequency stimulation at 130 Hz (or sometimes greater) was a popular stimulation protocol for the clinical treatment of Parkinson’s disease and essential tremor [3,11]. Repetitive high-frequency tetanic electric stimulation of different areas of brain, such as amygdala, hippocampus and cortex, could perform epileptic animal models. So in most studies of suppression of epilepsy the low-frequency electric stimulation was used. While high-frequency electric stimulation was also found the suppressive affects on epileptiform activity in rat hippocampal slices [2]. After 130-Hz electrical stimulation of the hippocampus the antiepileptic effect was observed in five patients with intractable temporal lobe epilepsy [6,13]. Some researchers reported that ECS suppressed epileptic and background activities in four patients with medically intractable focal epilepsy. They found that both high-frequency (50 Hz) and low-frequency (0.9 Hz) electric stimulation of the epileptic cortex suppressed interictal spikes. Furthermore, the effects of high-frequency stimulation were rapid and the effects of low-frequency stimulation were gradual [9]. We consider that the effect of high-frequency stimulation would induce the fast inactivation of sodium channels. For a very short time following an action potential a second stimulus can not excite a second action potential due to a phenomenon known as the absolute refractory period [8,2]. More studies would be needed to investigate the different effects and mechanisms of low- and high-frequency electric stimulation.

Taken together, this study showed that both low- and high-frequency ECS have the inhibitory effect on ferric chloride-induced seizures in rats, indicating their potential treatment of epilepsy in clinic.

Acknowledgements

We thank Dr. Jun Chen for technical assistance. This study was partially supported by Institute of Functional Brain Disorders, Fourth Military Medical University.

References