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Intensity-Dependent Effects of Mild Electric Foot Stimulation on Seizures in Chemical Kindling Model in Rats

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ABSTRACT

Introduction Epilepsy is a common neurological disorder that affects millions of people worldwide. While there are many treatment options available, including drug and non-drug therapies, there is still a need for effective treatments that can help manage seizures.The present study aimed to investigate the intensity-dependent effects of mild electric foot stimulation on seizure intensity following pentylenetetrazol (PTZ)chemical kindling in rats.

Methods: Kindled seizures were induced in rats by repeated injections of PTZ. Twenty-seven male rats were randomly divided into three groups: kindling group, kindling group + 0.1 mA electrical stimulation, and kindling group + 0.01 mA electrical stimulation. Electrical stimulation was induced using an electric box equipped with steel rods following acquisition of kindled seizures. The intensity of the mild electric foot stimulation was either 0.1 or 0.01 mA depending on the tested group.

Results: The study found that while mild electric foot stimulation with intensity of 0. 1 mA had proconvulsive effects on PTZ-induced kindled rats, and

decreased the latency to the onset of stage 5 seizure ($p<0.05$), stimulation with intensity of 0.01 mA did not have significant effects on seizure parameters.

Conclusion: Obtained results suggested that mild electric foot stimulation may have anticonvulsant effects, but only at certain intensity. This finding has important implications for future research into the use of mild electric foot stimulation as a treatment for epilepsy.

Keywords: Kindling; Epilepsy; Pentylenetetrazole; Electrical stimulation.

INTRODUCTION

Epilepsy is one of the most common chronic neurological disorders (1). People with epilepsy have uncontrollable (spontaneous) and frequent seizures that can be focal or generalized (2). Epilepsy has adverse effects on social, occupational, physical, and psychological functioning. Epilepsy was ranked as the third among chronic neurologic diseases in terms of disability-adjusted life years (3). Studies have shown the prevalence of epilepsy in about 1% of the world's population (1, 2). About one-third of epilepsy patients do not respond satisfactorily to antiepileptic drugs (4), even if several anticonvulsant drugs have been used alone or in different combinations. This phenomenon is called drug resistance (5). Among the complications of drug-resistant epilepsy, suicide and depression can be mentioned (6). Therefore, despite the availability of common drug treatments, it is necessary to discover new medical methods to help epilepsy patients.

Due to the unethical nature of research on patients, today various animal models are used to investigate the mechanisms of epilepsy as well as to identify drugs and epilepsy control methods. Among these models, drug seizure induction is a common method. This model is used to investigate the pathology of epilepsy (7). Pentylenetetrazole (PTZ) is widely used as a seizure-inducing drug in animals. PTZ is a GABA-A receptor antagonist. PTZ suppresses the function of inhibitory synapses and leads to increased neuronal activity. As a result, it causes general convulsions in animals (8).

Studies have shown that electrical stimulation can suppress seizure activity (9). Deep brain stimulation (DBS), which is applied to the anterior nucleus of the thalamus (10), cerebellum (11), and subthalamic nucleus (12) and vagus nerve stimulation (VNS) (13) are safe and effective treatments used to treat refractory epilepsy. However, the results obtained in our laboratory have shown that even mild electrical stimulation of the skin can control seizures (14). The results have shown that although mild electrical stimulation of the skin is effective in inhibiting the induction of epileptic seizures. However, it does not affect epilepsy. The purpose of this research was to re-evaluate the effect of the electrical stimulation intensity on the seizure intensity in an chemical kindling model of seizures.

MATERIALS ND METHODS

Twenty-seven male Wistar rats (200-250 g) were randomly assigned to three groups. The animals were housed in a 12-hour light-dark cycle at a temperature of 22 ± 2°C in the animal facility of the Department of Physiology at Arak University of Medical Sciences. Food and water were freely available to the rats, except during experimentation. The rats were kept in environmentally controlled conditions, with a 12-hour light-dark cycle (7:00-19:00 h light and 19:00-7:00 h dark), at a temperature of 22°C ± 2°C.

All experimental procedures were conducted in accordance with the standards of the university's ethics committee (Arak University of Medical Sciences Research Ethics Committee, ethical approval number IR.ARAKMU.AEC.1402.006).

Rats were randomly divided into three groups: kindling group (n=8), kindling group + 0.1 mA electrical stimulation (n=11), and kindling group + 0.001 mA electrical stimulation (n=8). The kindling group underwent a kindling process, which consisted of injecting subliminal doses of pentylenetetrazole (PTZ; 37.5 mg/kg) every 48 hours for 26 days. The kindling process was completed when seizures reached stage 5 after three consecutive days. After completing the kindling process, the rats were placed in the electrical stimulation box for 5 days without receiving electrical stimulation. In the electrical stimulation groups, the process was similar to the kindling group, except that the rats received mild electric foot stimulation (0.1 or 0.01 mA) three times a day for 5 days, starting 48 hours after completion of the kindling process.

Kindling

The kindling process was induced by injecting PTZ subliminally (37.5 mg/kg) every 48 hours for 26 days. Seizure severity was classified by Racine's scale using a five-stage scale (15): stage 0 = no response; stage 1 = shaking ears and face; stage 2 = myoclonic jerks; stage 3 = myoclonic jerks with upright posture and bilateral forelimb clonus; stage 4 = tonic-clonic seizure; stage 5 = generalized tonic-clonic seizure with loss of postural control.

Electrical Stimulation

Electrical stimulation was induced using an electric box equipped with steel rods (29 parallel rods, 0.3 cm diameter, spaced 1.0 cm apart). The intensity of the mild electric foot stimulation was either 0.1 or 0.01 mA depending on the tested group. Inevitable foot-shock (rectangular wave, 0.1 or 0.01 mA, 160 ms duration with a 160 ms interval for 20 min) was delivered to grid floor.

Data analyzing

Statistical analyses were performed using graphpad prism (Version 6). Data are presented as mean ± SEM. Paired Student's t-test (two-tailed) was used to compare the seizure parameters before and after treatments. Seizure stage data was analyzed by nonparametric Kruskal-Wallis

with post-hoc Mann Whitney U tests. P<0.05 was considered as the criterion for significance.

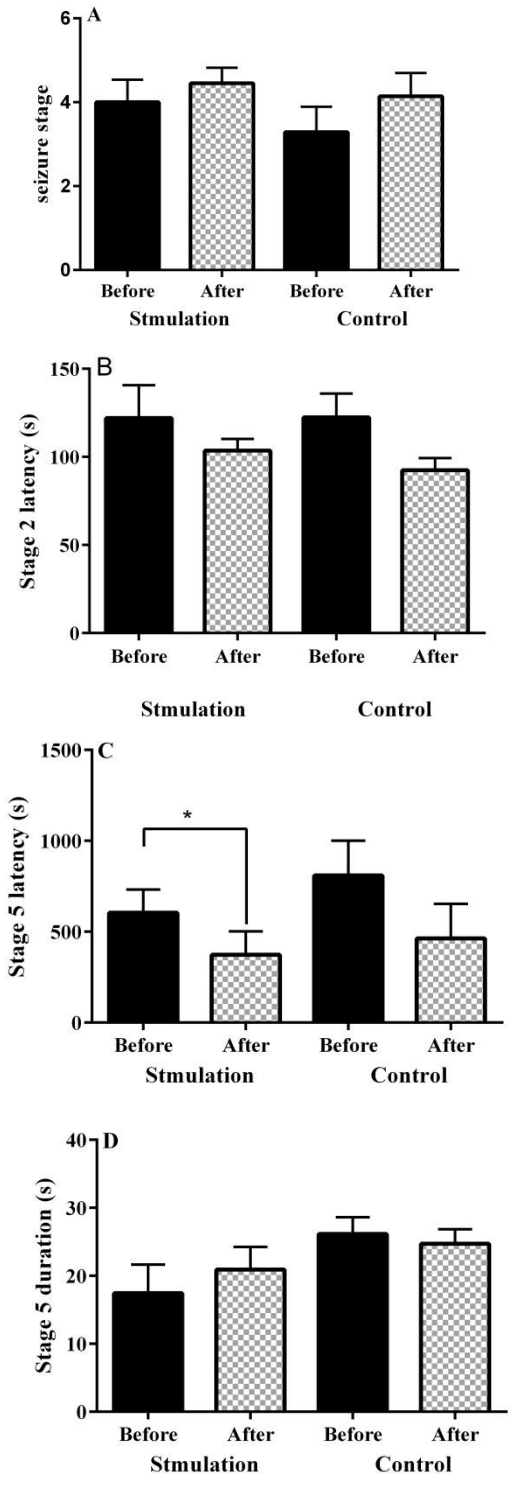

Figure 1: Effect of 0.1 mA electrical foot stimulation (EFS) on seizure stages (A) and stage 2 latency (B), stage five latency (C) and stage five duration (D) in pentylenetetrazole kindled rats. Stage five latency was reduced significantly after electrical foot stimulation. The values are shown as Mean ± S.E.M. * $p < 0.05$ compared to before electrical foot stimulation. Statistical analysis was performed by paired t-test.

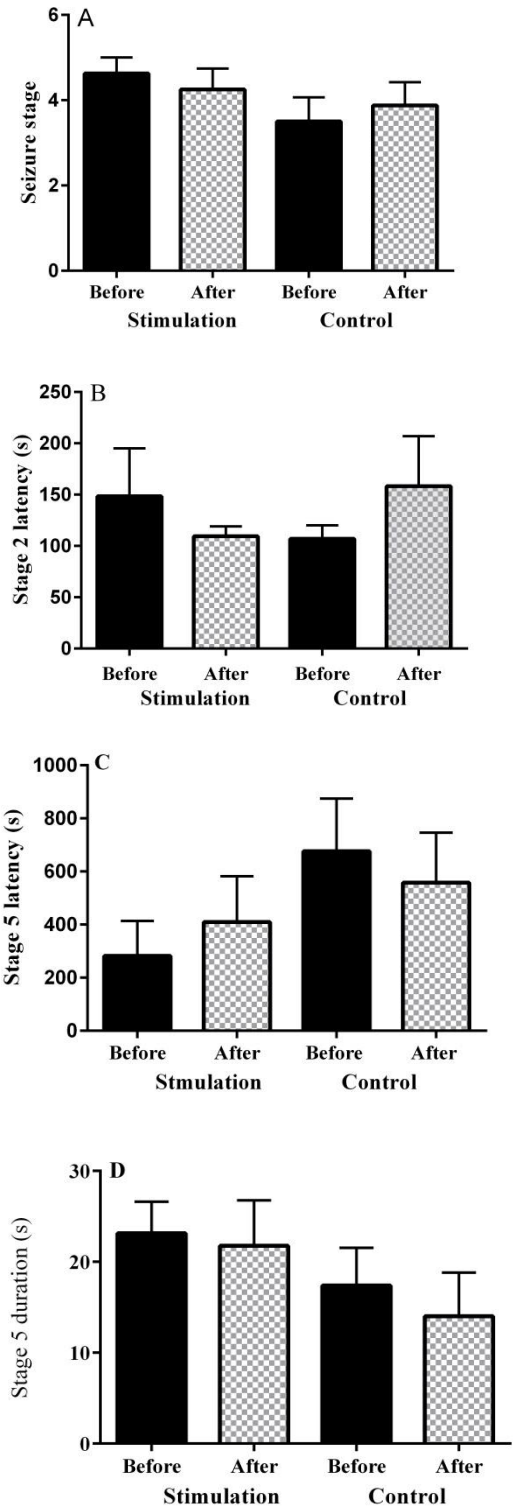

Figure 2: Effect of 0.01 mA electrical foot stimulation (EFS) on seizure stages (A) and stage 2 latency (B), stage five latency (C) and stage five duration (D) in pentylenetetrazole kindled rats. Statistical analysis using a paired t-test did not show significant differences between groups. The values are shown as Mean ± S.E.M.

RESULTS

Electrical stimulation of kindled rats for 5 days and 3 times a day with an intensity of 0.1 mA and a time interval of 40 minutes between simulations showed that this type of stimulation significantly reduces the time required for animals to reach stage five seizures ($P<0.05$) (Fig. 1C), that means mild electric foot stimulation has proconvulsive effect on PTZ kindled rats.

Comparison of other seizure parameters (S2L, S5D and seizure stage) before and after 0.1 mA mild electric foot stimulation showed that there was no significant difference between before and after mild electric foot stimulation (Fig. 1A, 1B, 1D).

Comparison of seizure parameters at intensity of 0.01 mA showed that mild electric foot stimulation with this intensity had no significant effect on any of the seizure parameters (Fig. 2A, 2B, 2C and 2D).

DISCUSSION

The results of this study demonstrate that mild electric foot stimulation with an intensity of 0.1 mA did not prevent seizures, but rather exacerbated some seizure parameters. This finding suggests that simply reducing the intensity of electrical stimulation may not be sufficient to achieve anticonvulsant effects.

Many experimental and clinical efforts have been made to control epileptic seizures using physical factors such as electric stimulation (14, 16). The use of physical stimuli in many cases has had positive effects on seizure control. Electrical stimulation is a non-invasive and non-pharmacological physical stimulus. At the cellular level, electrical stimulation can interact with a variety of cellular components, such as ion channels, membrane-bound proteins, cytoskeleton and intracellular organelles (17). At the molecular level, it can facilitate the transport of both charged and uncharged biomolecules through biological membranes via electrophoresis and electroosmosis (18). Due to these direct effects on biomolecules and cells, electrical stimulation has been utilized in a wide range of biomedical and clinical applications. However, the results of this study suggest that electrical stimulation may not be effective in all cases. Results showed that the current intensity used in electrical stimulation can be one of the important parameters in seizure control. In such a way that reducing the intensity of current can cause procovulsive effect in rats instead of inhibition of seizure. In confirmation of these results, data published by Bortel et al. In 2019, has shown that electrical stimulation of the skin can cause seizures in rats (19). Also Deonna et al. in 1998 reported that electrical stimulation of the peripheral nerves can induce somatosensory-evoked seizures (20). So it seems that the results of this study suggest that reducing the intensity of electrical stimulation did not inhibit the induction of seizures and may even have increased some convulsive parameters. Therefore, it seems that reducing the intensity of electrical stimulation may not be a suitable way to reduce the stressful effects of this type of treatment. Instead, changing other stimulation parameters such as stimulation wave frequency or stimulation duration may be a more appropriate way to inhibit chemical kindling with PTZ.

CONCLUSION

The results of this study suggest that mild electric foot stimulation may have anticonvulsant effects, but only at certain intensity. This finding has important implications for future research into the use of electrical stimulation as a treatment for epilepsy.

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ETHICAL APPROVAL

Ethical approval for the study was provided by the Arak University of Medical Sciences Research Ethics Committee # IR.ARAKMU.AEC.1402.006.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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