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Relationship Between Response Threshold to Electrical Stimulation and Sensitivity to Pentylenetetrazol Kindling in Male Rats

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Abstract

Background and Objective: Previous studies have demonstrated that foot electrical stimulation has the potential to inhibit kindling development in rats. However, it remains unclear whether there is a relationship between the sensitivity to electrical stimulation and its inhibitory effect on kindling. Therefore, the aim of this study was to investigate the correlation between chemical kindling and electrical properties of the rats' paw.

Materials and Methods: Thirty-two male Wistar rats were divided into three groups; control, 24 hours after kindling and 60 days after kindling. Pentylenetetrazol (PTZ) kindling was induced by injecting PTZ (37.5 mg/kg). Response threshold to electrical stimulation and paw electrical resistance was measured in the three groups.

Results: The findings showed that there is a positive correlation between stage 5 duration and response threshold to electrical stimulation ($p<0.05$) and a negative correlation between stage 5 latency and the response threshold to electrical stimulation ($p<0.05$). Measuring the paw resistance showed that while in the control rats there was no significant correlation between the response threshold to electrical stimulation and the plantar electrical resistance, in rats that had 60 days passed since their chemical kindling, there was a significant and positive correlation ($p<0.05$).

Conclusion: The data suggest that rats that had a higher threshold for electrical stimulation have a faster, onset and longer duration for seizures induced by PTZ. Furthermore, the comparison of paw resistance revealed the potential impact of kindling on the relationship between paw electrical properties and seizure susceptibility.

Keywords: Pentylenetetrazol Kindling, Response Threshold, Electrical Resistance

1. Introduction

Epilepsy is a prevalent neurological disorder. This disease, is a brain disorder characterized by a lasting predisposition to produce seizures (1). Epileptic seizures and associated comorbidities such as cognitive deficits and intellectual disability arise when certain threshold levels are exceeded, which depend on a given individual's diathesis or susceptibility (2). This susceptibility is influenced by complex interactions between genetic and environmental factors and can vary over different timescales, including daily fluctuations, developmental changes, and aging, among others (3). It is known that in "normal" tissue, the seizure threshold is higher, making it more difficult to trigger seizures compared to chronic epilepsy Thus, it is possible to hypothesize that

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the threshold is decreased in epilepsy (there is a higher likelihood of seizures in epilepsy patients compared to the general population) (4). Studies have shown that in comparison to nonvulnerable animals, vulnerable animals exhibited significantly accelerated epileptogenesis and a lower threshold to attain status epilepticus. Hence, certain animals have altered diathesis due to an unresolved previous history of stress, making them more prone to developing epilepsy (5).

A specific inhibitor of the gamma-aminobutyric acid A (GABAA) receptor is pentylenetetrazole (PTZ). It operates on chloride channel-coupled receptors (6). In an animal model of epilepsy, PTZ was utilized to cause chemical kindling. This type of modeling, which is performed in the study of human epilepsy, describes a phenomenon in which repeated PTZ injections gradually induce the development of seizures, which end in generalized tonic-colonic seizures. The process of PTZ-induced kindling occurs in two stages. The first stage, known as epileptogenesis, is characterized by progressive convulsant activity that results from repeated PTZ administrations. And the second stage the established epileptic state, or the stage at which seizures classified as stage five are recognized, is the second phase (7).

Electrical stimulation has been used in numerous clinical and experimental attempts to control epileptic seizures (8). Numerous modalities of electrical stimulation have been proposed for the treatment of epilepsy, including deep brain stimulation applied to the anterior nucleus of the thalamus, cerebellum, and subthalamic nucleus, as well as spinal cord stimulation and also foot electrical stimulation (9). Previous research has shown that foot electrical stimulation inhibits the development of kindling in rats, but it has no effect on the kindled animals (7). It is possible that this difference in the effect of electrical stimulation in inhibiting kindling is caused by the difference in the animal's response to electrical stimulation. The goal of this study was to investigate the association between the response threshold to foot electrical stimulation and the susceptibility to PTZ kindling in male rats.

2. Material and Method

2.1. Animals

A total of 32 male Wistar rats (weight = 200-250 g) were used in this study. Rats were bred and raised from the animal house of Arak University of Medical Sciences. Rats were classified in 3 groups as follows: Group 1 (n = 8), the animals received saline. Group 2 (n = 13), the animals were newly kindled with PTZ. Group 3 (n = 11), the animals were kept after kindling for 2 months. The rats were kept in environmentally controlled conditions (12 h light–dark cycles, 7:00–19:00 light and 19:00–7:00 dark, temperature 22±2 °C) at the Arak University of Medical Sciences animal facility. Water and food were abundantly available. All experimental procedures were conducted in compliance with the regulations outlined in the EU Directive 2010/63/EU and adhered to the ethical standards set by the local ethics committee (Arak University of Medical Sciences Research Ethics Committee # IR.ARAKMU.AEC.1402.006).

2.2. Kindling

For induction of kindling, a sub-convulsive dosage of PTZ (37.5 mg/kg, intraperitoneal injection, Sigma, USA) was administrated 13 times at 48-hour intervals for a duration of 26 days. The occurrences of seizure behaviors and their durations were recorded during kindling development and the different kindling stages were classified. The following were their parameters: phase zero involves no reaction; phase one involves twitching of the ear and face; phase two involves myoclonic jerks without upright posture; phase three involves myoclonic jerks with upright posture combined with bilateral forelimb clonus; phase four involves tonic-clonic seizures; and phase five involves generalized tonic-clonic seizures and loss of postural control.

2.3. Measurement of threshold and resistance

Foot electrical stimulation was performed in this study. A box (30 cm x 30 cm x 40 cm high) with a steel-rod floor (29 parallel rods, each 0.3 cm in diameter and spaced 1.0 cm apart) was used to induce electrical stimulation. For measuring the response threshold to electrical stimulation, rats individually received foot electrical stimulation with a frequency of 3 Hz and the initial current intensity was set at 0.1 milliamperes for 20 seconds, and the current intensity was subsequently increased in steps of 0.1 milliamperes until the rat exhibited a noticeable sign of shock. For measuring the electrical resistance, Rats were placed on a metal grid, and voltmeter electrodes were placed on the grid between 5 and 10 cm from the animals. The electrodes were abandoned to stabilize for a few seconds. so that steady readings could be obtained.

2.4. Statistical analyses

Statistical analyses were performed using GraphPad Prism Statistics (Version 6). Data are presented as mean ± SEM. Correlation analysis was performed using Pearson Product-Moment Correlation.

3. Results

3.1. The correlation between the response threshold to electrical stimulation and the susceptibility in control groups

Correlation analysis between seizure parameters and the response threshold to foot electrical stimulation of the in three groups showed that control animals and rats whose kindling was completed 24 hours before did not show significant correlation between seizure parameters and response threshold to foot electrical stimulation. For rats that have been kindled for 60 days, there was a significant and positive correlation between seizure parameters and response threshold to foot electrical stimulation (Figure 1). Statistical analysis showed that in this group, there was a significant and positive correlation between S5 duration and response threshold ($p<0.05$) and significant negative correlation between S5 latency and response threshold to foot electrical stimulation ($p<0.05$). There was not significant correlation between other seizure parameters such as seizure stage and S2 latency and response threshold to foot electrical stimulation in this group.

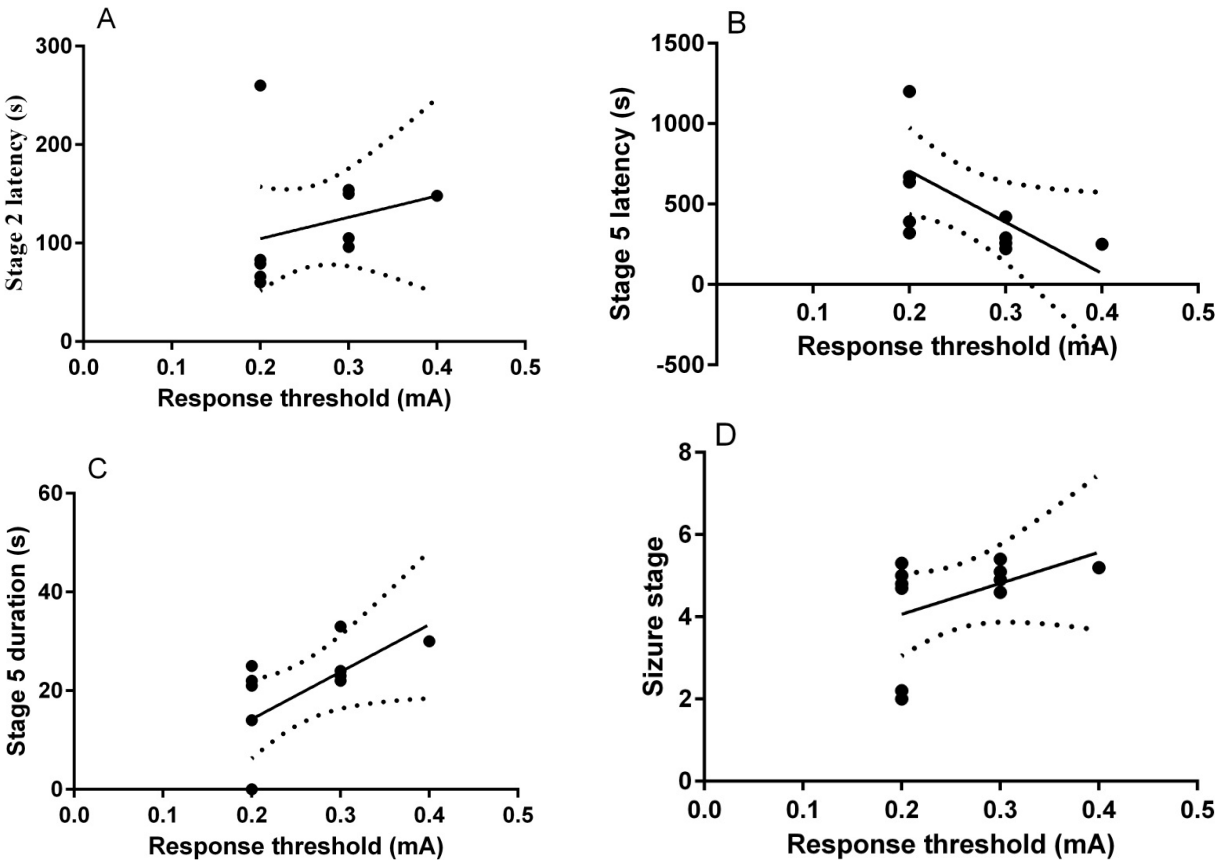

Fig. 1. The correlation between the response threshold to electrical stimulation and seizure parameters in rats that have been kindled for 60 days. Statistical analysis showed that there was a significant and positive correlation between S5 duration and response threshold ($p<0.05$, C) and a significant negative correlation between S5 latency and response threshold to foot electrical stimulation ($p<0.05$, B). There were not significant correlations between other seizure parameters such as seizure stage (D) and S2 latency (A) and response threshold to foot electrical stimulation.

Measuring the plantar resistance in control group and comparing it with rats in the other two groups showed that while in the control rats and rats that kindling were completed in the last 24 hour,

there was no significant correlation between the response threshold to electrical stimulation and the plantar electrical resistance, but in the rats that 60 day had passed since their chemical kindling, there was a significant and positive correlation ($p<0.05$) between plantar resistance and response threshold to foot electrical stimulation (Figure 2).

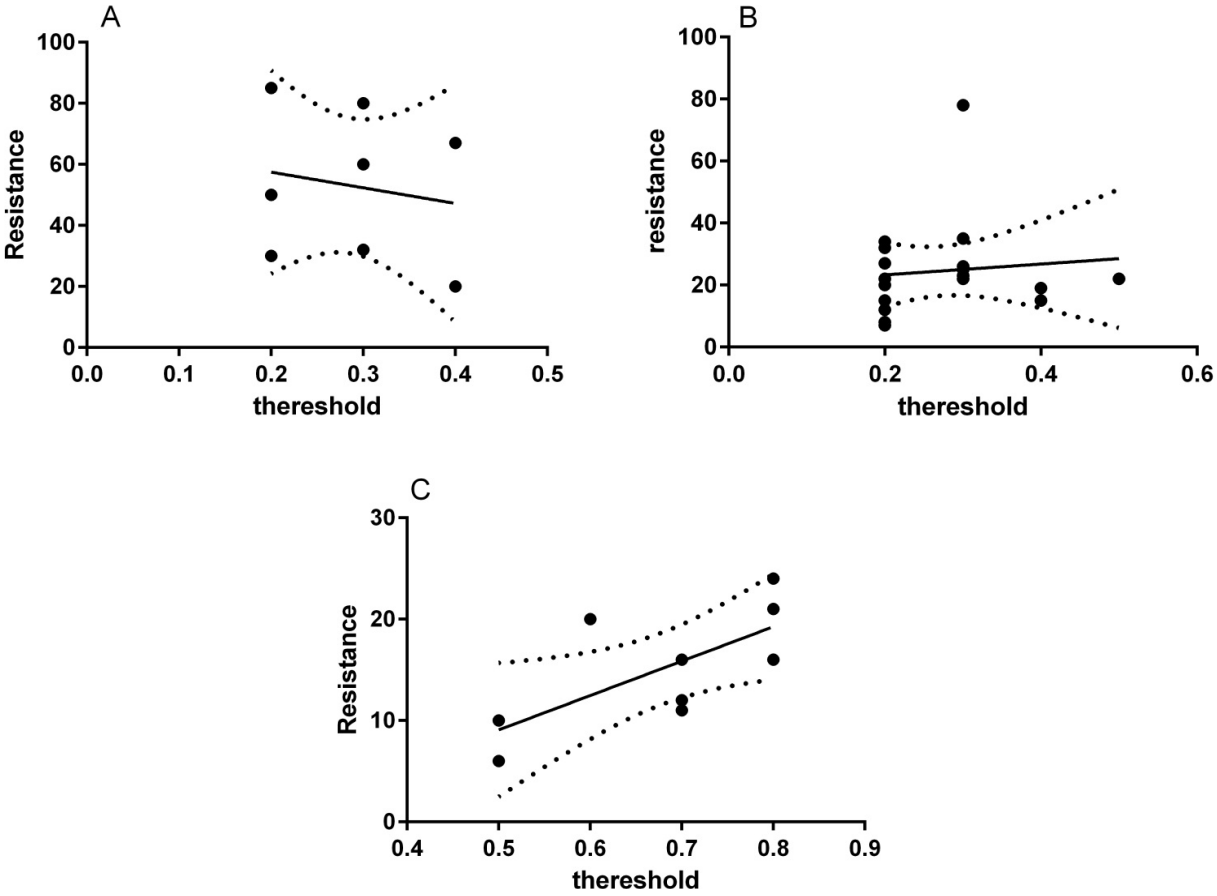

Figure 2. The correlation between the response threshold to electrical stimulation and paw electrical resistance control (A), rats that kindling were completed in the last 24 hour (B) and in rats that have been kindled for 60 days (C). As has been shown in the figure, only rats that have been kindled for 60 days have positive and significant correlation between paw electrical resistance and response threshold to electrical stimulation ($P<0.05$).

4. Discussion

The results of this research showed the in the rats that 60 days had passed since their chemical kindling, significant and positive correlation between plantar resistance and response threshold to foot electrical stimulation.

Previous data have revealed the effect of foot-shock stress on inhibiting kindling seizures (10). There has been a lot of research on anticonvulsant effect of electrical stimulation (electroshock) on epilepsy models (7, 11-13). In these researches, electric stimulation with an intensity of about 0.2 mA was delivered to the animal (13, 14). But whether these stimulations have the same effects in all animals or not is a question that has not been answered yet. It has been shown that slow kindled rats exhibited a greater startle reflex to noise relative to fast kindled rats (15). As like as these data, the results of this research showed that rats that showed a higher response threshold to electrical stimulation have a higher susceptibility to convulsions induced by pentylenetetrazole. This relationship was not seen in the control rats (non-Kindled) and rats that have passed 48 hours since the time of kindling. This issue can be caused by the changes that occur within two months after

kindling in the structure of the skin or brain and spinal cord of kindled rats.

Although previous researches was based on the concept that electrical stimulations by affecting the synchronization of action potentials in neurons or changes in the threshold of the neurotransmitter release cause resistance to convulsions in animals (14), our results showed that changes in the electrical properties of the skin can also be effective in this phenomenon. Also, these results may be due to the fact that rats that need higher currents to respond to electrical stimulation are more resistant to stress caused by repeated injections of pentylenetetrazol. Previous results have shown that repeated stress can reduce the animal's response to kindling (16, 17). Therefore, it seems that the lack of sensation of electrical stimulation and the reduction of the effect of stress in animals can cause the exacerbation of seizures in them. Although some studies have shown that repeated stress accelerates the progression of kindling (18). It seems that the intensity of stress is an important factor in such a way that the intensity of stress should be so much that it can cause the release of the corticosterone hormone in a stable way (that is, the electric intensity of about 2 mA), while in this research, the intensity of the stimulus was much less than this value (0.6 mA).

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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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