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Improving effect of mild foot electrical stimulation on pentylenetetrazole-induced impairment of learning and memory☆

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ABSTRACT

Epilepsy is a common neurological disorder that affects learning and memory. Recently it has been shown that mild foot electrical stimulation (MFES) can increase learning and memory in normal rats. Pentylenetetrazole (PTZ) kindling is a model of human epilepsy. As with human epilepsy, PTZ kindling impairs learning and memory in rats. The purpose of this study was to investigate the effect MFES on kindling-induced learning and memory deficits in rats. Forty-nine male Wistar rats weighting 200 to 250 g were divided into the following seven groups: PTZ only, phenytoin only, MFES only, PTZ plus phenytoin, PTZ plus MFES, phenytoin plus MFES, and saline (control), with the treatments administered for 26 days. Forty-eight hours after the last injection, the animals performed the Morris water maze (MWM) task, and spatial learning and memory were measured. The results indicated that although chronic administration of phenytoin inhibited the development of PTZ kindling, it did not exert a protective effect against kindling-induced spatial learning and memory impairment in rats. On the other hand, pretreatment of PTZ-kindled animals with MFES significantly improved spatial working and reference memory. The results point to potential novel beneficial effects of MFES on learning and memory impairment induced by PTZ kindling in rats.

1. Introduction

Epilepsy, which is characterized by recurrent seizures, is a common neurological disorder, affecting approximately 1% of the world's population [1]. Previous research demonstrated an association between epilepsy and cognitive impairment, such as impaired learning and memory [2]. Although antiepileptic medications can prevent the occurrence of epileptic seizures in many cases, cognitive impairment has been reported to be a side effect of antiepileptic drugs. For example, impairments in learning and memory were reported after administration of phenytoin in normal [3] and pentylenetetrazole (PTZ)-kindled rats [4]. Thus, the discovery of new methods capable of controlling the side effects of seizures is vital.

Kindling is an animal model of epilepsy. It is produced by repeated application of electrical or chemical stimulation, leading to the development of seizure activity and culminating in an epileptic-like condition [5,6]. Pentylenetetrazole is a chemical agent used to induce chemical kindling by blocking Cl^- channels associated with GABAA receptors [7]. Kindling-induced cognitive impairments have been reported with both chemical [8] and electrical kindling [9]; PTZ kindling profoundly affects the hippocampus, which is a vital structure for the acquisition of new declarative memories [10].

Many types of behavioral assays have been used to examine spatial memory following kindling [11,12]. The Morris water maze (MWM) is a test of hippocampus-dependent spatial learning and memory in rodents that is the closest parallel to episodic memory in humans [13]. Previous studies provided evidence of impaired episodic and spatial memory in humans, as well as in rats in the MWM, after epilepsy [14] or induced seizures [15].

According to recent evidence, mild foot electrical stimulation (MFES) improved learning and memory in rats [16,17]. It has been reported that after MFES, the number of cells in the CA1 region and gyrus dentatus and hippocampal increased, and there is a strong positive correlation between the cell number of CA1 region and gyrus dentatus and the time spent on the target quadrant in the MWM [18]. However, the effect of MFES on the prevention of deficits in learning and memory after kindling has not been examined. The aim of this study was to investigate the effect of MFES on kindling-induced learning impairment and compare it with that of phenytoin.

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2. Material and methods

2.1. Animals and housing conditions

Forty-nine male Wistar rats weighing 200 to 250 g were used in this experiment. The animals were housed under environmentally controlled conditions (four animals per cage) (12-h light/dark cycles, 07:00–19:00 h light and 19:00–7:00 h dark, temperature between 22 ± 2 °C) at the Arak University of Medical Sciences animal facility. Food and water were supplied ad libitum. All procedures were carried out in accordance with EU Directive 2010/63/EU, as well as with all norms established by the local ethics committee (Arak University of Medical Sciences Research Ethics Committee: # 94-132).

2.2. Experimental groups

The rats were randomly divided into the following seven groups, with seven rats in each group: 1- control group treated with 0.5 ml of saline (intraperitoneally [i.p.] every second day); 2- PTZ group treated with 37.5 mg/kg (i.p. every second day); 3- MFES group (MFES treatment every second day); 4- phenytoin group treated with 30 mg/kg of phenytoin (i.p. every second day); 5- PTZ plus foot stimulation group treated with MFES 60 min before PTZ injections every second day; 6- PTZ plus phenytoin group treated with 30 mg/kg of phenytoin i.p. 60 min before a PTZ injection every second day; and 7- foot stimulation plus phenytoin group treated with 30 mg/kg of phenytoin i.p. 60 min before MFES every second day. This protocol was administered to the animals for 26 days. The animals were then introduced to the MWM experiment.

2.3. Behavioral procedures

2.3.1. Kindling

For kindling, a subthreshold dose of PTZ (37.5 mg/kg, i.p. Sigma, USA) was injected i.p. every 48 h for 26 days (13 injections). Immediately following the injection, the convulsive behavior of each subject was observed for 20 min, and convulsive behavior was recorded for 30 min. The seizure intensity was classified using the following scale: stage 0 = no response; stage 1 = ear and facial twitching; stage 2 = myoclonic jerks, without an upright position; stage 3 = myoclonic jerks, an upright position, and bilateral forelimb clonus; stage 4 = tonic–clonic seizures; and stage 5 = generalized tonic–clonic seizures and loss of postural control [5]. Rats were considered kindled when seizure attacks (stage 5) occurred after each PTZ injection for three consecutive days. The following parameters were recorded: seizure stage, latency to onset of stage 2 and stage 5 seizures, and stage 5 duration.

2.3.2. Foot electrical stimulation

Electrical stimulation was induced using a box (30 cm × 30 cm × 40 cm high) with a steel-rod floor (29 parallel rods, 0.3 cm in diameter, set 1 cm apart). Foot shock (rectangular electrical stimulus of 0.2 mA, duration of 160 ms, intervals of 160 ms) was delivered to the grid floor for 20 min. Mild foot electrical stimulation is continued during kindling procedure and MWM learning.

2.3.3. MWM test

Morris water maze test was performed 48 h after the last injection of PTZ and 1 h after MFES. The water maze consisted of a large circular black pool, with a diameter of 140 cm and height of 60 cm. A cylindrical shaped escape platform (10 cm in diameter) was placed 30 cm from the wall. The pool was filled with water (22 °C) to a depth of 1.5 cm below the surface of the escape platform. The temperature in the testing room was maintained at 22 °C and contained several extravisual cues. The movements of the rat in the water maze were monitored using a video camera placed above the pool. Output from the video camera was fed into a computer, with handmade analytical software based on matlab program image processing used to measure the total distance traveled, average swimming speed, and time spent in each quadrant of the maze.

The rats performed four trials a day on 4 consecutive days. At the start of each trial, the rat was placed in the water, so that it faced the wall. The rat then swam until it reached the platform. If the rat found the platform, it was left on the platform for 20 s before being removed for an intertrial interval of 10 min. If the rat did not find the platform after 60 s, it was gently guided to the platform and allowed to remain on the platform for 20 s. To assess spatial memory retention following a period of learning, probe trials were conducted 24 h after the last hidden platform training. During the probe trials, the platform was removed, and each animal was allowed to swim for 60 s starting from the same position to evaluate time the animals spent in the target versus that of other quadrants.

2.4. Corticosterone measurement

Animals were sacrificed by decapitation 1 h after the last behavioral test, which always took place between 15:00 h and 16:00 h. Blood was collected, and serum corticosterone levels were evaluated. Plasma corticosterone was measured by commercial RIA kits (DRG, Germany). A radioimmunoassay was performed according to the manufacturer's instruction.

2.5. Data analysis

Data are presented as mean ± S.E.M. Unpaired *t*-tests were conducted for comparisons of two independent groups. Comparisons of latencies to find the platform in the various treatment groups were analyzed with a one-way or two-way analysis of variance (ANOVA), followed by Tukey's test. Slope of learning was determined by plotting the learning latencies and obtaining the slope of the linear regression, and slope of learning was compared using Pearson's correlation coefficient. Differences were considered significant at the level of $p < 0.05$.

3. Results

The results of the two-way ANOVA of average learning curves in the control, PTZ, and MFES groups following 4 days of training in the MWM showed a significant difference between groups ($F(3, 60) = 24.57$, $p < 0.0001$; two-way ANOVA). The post hoc analysis using Tukey's test showed that phenytoin did not have a significant effect on learning acquisition in the MWM test. However, PTZ significantly decreased learning ability, and MFES significantly increased learning ability in the test as compared with that of the controls (PTZ: higher latencies on day 4, $p < 0.05$; MFES: lower latencies on days 2 and 3; both $p < 0.01$), as shown in Fig. 1A. We estimated the slope of learning by fitting a line to the escape latency. Statistical analysis of the slope of learning using Pearson's correlation coefficient pointed to a trend toward significantly lower slope values in the phenytoin-treated group as compared with those of the control group over days ($z = -2.24$, $p = 0.025$), as shown in Fig. 2. Statistical analysis with two-way ANOVA showed that Swim speed did not differ between groups ($F(3, 20) = 2.84$, $p > 0.05$, Fig. 1B).

A probe trial was performed on day 5 (i.e., after 4 days of training) to further assess spatial memory. The results of a one-way ANOVA of the data revealed a significant difference between the groups ($F(3, 20) = 23.19$, $p < 0.0001$). The post hoc analysis using Tukey's test showed that the time spent in the training (platform) quadrant was significantly decreased in the phenytoin-treated ($p < 0.0001$) and PTZ-treated ($p < 0.0001$) groups but unchanged in the MFES-treated group (Fig. 3).

Fig. 4A shows the effect of MFES on PTZ-induced learning disability. As apparent in the figure, MFES significantly reduced the time ($p < 0.05$)