

The effects of treadmill exercise on penicillin-induced epileptiform activity

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Abstract

Introduction: The aim of this study was to evaluate the effects of short-, moderate- and long-duration treadmill exercise (15, 30 and 60 min) on the mean frequency and amplitude of penicillin-induced epileptiform activity in rats.

Material and methods: In this study, 32 rats were assigned to 15, 30, and 60 min running exercise groups and the control group, each consisting of 8 rats. According to the specified protocol, the rats were submitted to running exercises at the same hour of each day for 90 days. After the exercise program, the rats were administered (500 IU/2.5 μ l) of penicillin into the left cortex by the microinjection method. An electrocorticogram (ECoG) recording was performed for 3 h using a data acquisition system. The frequency and the amplitude of the recordings were analyzed.

Results: Short-duration treadmill exercise (15 min) caused a decrease in the frequency of penicillin-induced epileptiform activity at 70 min after penicillin injection ($p < 0.001$). The mean frequency of epileptiform activity decreased at 90 min after penicillin injection in the 30 and 60 min treadmill exercise groups ($p < 0.01$). The mean amplitude of epileptiform activity was not changed in any of the exercise groups compared to the control ($p > 0.05$).

Conclusions: The results of the present study demonstrate for the first time that short-, moderate- and long-duration treadmill exercises decreased the frequency of penicillin-induced epileptiform activity. These findings may contribute to improving the quality of life in epileptic patients.

Key words: epilepsy, seizure, rat, treadmill exercise.

Introduction

The importance of regular physical exercises to one's health becomes increasingly evident with each passing day. Although the favorable effect of physical fitness on general health is unquestionable, physical exercise and fitness programs in patients with epilepsy are still a matter of controversy [1]. Epilepsy is one of the most common serious disorders of the brain [2–4]. It affects at least 50 million people worldwide; 80% of them are in the developing world, where 80–90% of people are believed to receive inadequate or no treatment at all [3, 5].

In the evaluation of the relationship between exercise and epilepsy, it appears that epileptic patients most often avoid involvement in physical activities and maintain a rather sedentary life. The underlying reason, for both patients and doctors, is the belief that physical activity might cause injuries in epileptic patients and the fear of escalation of epileptic seizures [6].

Although the putative positive or negative effect of physical exercise on the frequency of seizures seems to be an unanswerable question, it is expected to show favorable effects on maximum aerobic capacity, working capacity, and body weight in the patient group as it does in healthy individuals. Unfavorable influences have been observed on general well-being and the quality of life in epileptic patients who abstain from physical exercise. There are, however, various clinical and experimental studies [7–10] indicating that regular sport activities or physical therapy programs are beneficial for patients with convulsive disorders. Epileptic patients performing regular exercises feel better, have better seizure control, and show a marked decline in the number of seizures [7–10].

Running and walking are normal behaviors of rodents [11], and therefore treadmill exercise was considered to be an appropriate exercise model. In today's practice, the treadmill exercise test is commonly used in human and animal studies to evaluate different functions and capacities [12–15].

The aim of this study was to determine the impact of different intensities of treadmill exercise on the brain's susceptibility to penicillin-induced epileptic activity. For this purpose, the effects of treadmill exercises (15, 30, and 60 min/day for 90 days) on penicillin-induced epileptiform activity in adult rats was investigated in this study. Penicillin was used to induce epileptiform activity in rats; it is a widely used method for inducing epileptiform activity by applying penicillin to the cerebral cortex [16]. The application of penicillin to the neocortex results in synchronous discharge of neurons, which bears an electrophysiological resemblance to human focal interictal epileptiform discharges [17].

Material and methods

The study protocol was approved by the Experimental Animal Ethics Committee of Ondokuz Mayıs University (2012/36). Thirty-two 20–24-week-old, male Wistar albino rats weighing 280–350 g were supplied by the Medical and Surgical Research Center of Ondokuz Mayıs University Faculty of Medicine. The CE-certified four-lane animal treadmill (May Time 0804, Animal Treadmill) with adjustable settings for rate, distance, running time, speed, inclination and a built-in memory to store data was used for experiments. In order to avoid any stress that may possibly arise in the course of the physical exercise, all rats were subjected to conditioning exercise at the lowest treadmill speed lasting 5 min over a period of 14 days and were randomly divided into four groups: the control group ($n = 8$), 15 min exercise group ($n = 8$), 30 min exercise group ($n = 8$) and 60 min exercise group ($n = 8$). To prevent avoidance and

force the animals, incremental electrical shock were given (1–6 mA) to continue running exercise.

After the treadmill adaptation period, control group rats were put on hold in the cages with the same conditions until the surgery, and experimental groups continued to exercise for 13 weeks according to the treadmill exercise protocol by Rico *et al.* [18]. This protocol consists of short- (15 min), moderate- (30 min) and long-duration (60 min) running exercise for 5 days per week (from Monday to Friday) between 10:00 and 12:00 AM for 90 days. In the first 2 weeks, all experimental groups were exercised for 15 min, and then the time was increased from 15 min to 30 and 60 min for two groups. The initial treadmill speed was 17 cm/s, with a grade inclination of 5°. Treadmill speed was gradually increased to 45 cm/s with increments of 2.5° in the grade incline to reach a final grade inclination of 18°.

ECoG recordings and surgical procedure

Rats were anesthetized with urethane (1.25 g/kg, *i.p.*) and placed in a stereotaxic frame under spontaneous respiration. Incision regions were infiltrated with prilocaine hydrochloride to prevent possible occurrence of pain. After shaving the top of the head, a 3 cm incision was created on the skull in the rostro-caudal direction. The soft tissue on the skull was removed and the bregma (reference point) was identified. Under stereotaxic guidance, two stainless steel screws were placed over the left somatomotor cortex (first screw 3 mm lateral and 4 mm rostral to bregma; second screw 3 mm lateral and 4 mm caudal to bregma) and a well conductor bipolar electrode was connected to the screws [19, 20]. After observing the brain's basal activity with the PowerLab data acquisition system, a 1 mm hole was opened (1.5 mm left lateral and 1.5 mm caudal to the bregma) with a hand drill for injection of penicillin G potassium (Sigma Chemical Co., St. Louis, MO, USA), which was dissolved in sterile physiological saline. The epileptic focus was produced by (500 IU/2.5 μ l) intracortical penicillin G potassium injection (1 mm vertical direction to the brain surface) using a Hamilton microsyringe (type 701N, 22s gauge, bevel tip) with a volume of 2.5 μ l. The drug dosage was determined according to Kozan *et al.* [21] and Aslan *et al.* [22].

Statistical analysis

All results are presented as the means \pm standard error of the mean (SEM). Statistical comparisons were made using the SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA). The normality of the data was tested with the one-sample Kolmogorov-Smirnov test before analyses. After verifying that data from electrophysiological recordings were normally distributed, one-way

analysis of variance (ANOVA) and Tukey-Kramer post hoc tests for multiple comparisons were performed. For all statistical tests, $p < 0.05$ was considered statistically significant.

Results

A single intracortical injection of penicillin (500 IU/2.5 μ l dose) induced epileptiform activity approximately 5 min after injection; the activity reached a constant level by 30 min after penicillin administration. Signs of epileptiform activity persisted for approximately 3 h. The means of the spike frequency and amplitude of the epileptiform activity were 49.74 ± 16.78 spike/min and 930 ± 150 μ V, respectively (Figure 1 A).

Short-duration treadmill exercise (15 min) caused a significant decrease in the mean frequen-

cy of epileptiform activity at 70 min after penicillin injection without changing amplitude compared to the control group; this remained significant until the end of the experiment ($p < 0.001$) (Figure 2). The mean spike frequency and amplitude of epileptiform activity were 21.26 ± 12.7 spike/min and 950 ± 400 μ V in the 15 min treadmill exercise group, respectively (Figure 1 B). The moderate- (30 min) and long-duration treadmill exercise (60 min) significantly decreased the mean frequency of epileptiform activity at 90 min after penicillin injection ($p < 0.01$) without changing amplitude compared to the control group (Figure 2). The mean spike frequency and amplitude of epileptiform activity were 30.69 ± 10.7 and 34.37 ± 17.6 spike/min and 880 ± 210 and 820 ± 200 μ V in the 30 and 60 min treadmill exercise groups, respectively (Fig-

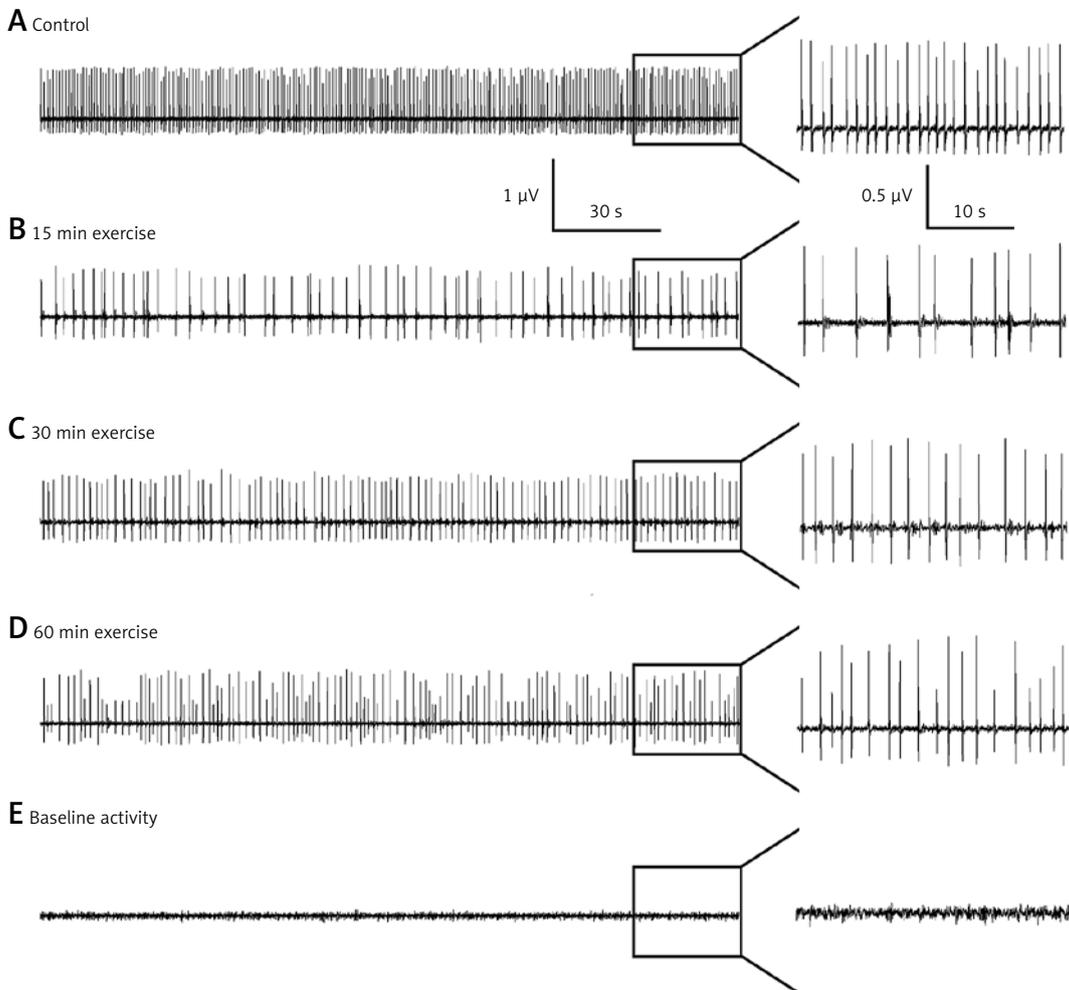


Figure 1. **A** – The intracortical injection of penicillin (500 IU/2.5 μ l) induced epileptiform activity on ECoG. **B** – The mean frequency of penicillin-induced epileptiform activity significantly decreased at 70 min after penicillin injection in the 15 min treadmill exercise group (treadmill exercise 15 min/day, for 90 days). **C** – The mean frequency of penicillin-induced epileptiform activity significantly decreased at 90 min after penicillin injection in the 30 min treadmill exercise group (treadmill exercise 30 min/day, for 90 days). **D** – The mean frequency of penicillin-induced epileptiform activity significantly decreased at 90 min after penicillin injection in the 60 min treadmill exercise group (treadmill exercise 60 min/day, for 90 days). **E** – The injection of normal saline (2.5 μ l, *i.c.*) did not alter the mean frequency or amplitude of penicillin-induced epileptiform activity. Representative ECoGs are presented for 100 min after penicillin administration

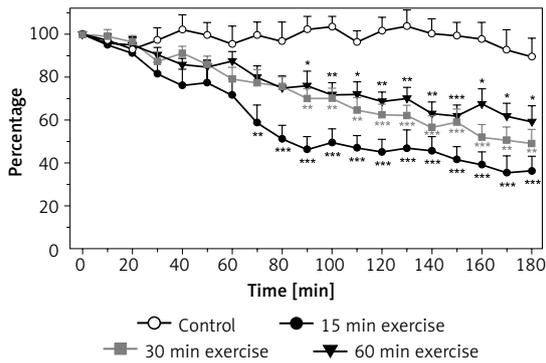


Figure 2. The effects of treadmill exercise on the mean spike frequency of penicillin-induced epileptiform ECoG activity. The mean frequency of ECoG epileptiform activity significantly decreased at 70 min after penicillin injection in the 15 min treadmill exercise group. The mean frequency of ECoG epileptiform activity significantly decreased at 90 min after penicillin injection in the 30 and 60 min treadmill exercise groups. The groups were compared by one-way ANOVA followed by the Tukey-Kramer post hoc test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ indicate significant differences compared to control group. The percentage frequency of epileptiform ECoG activity value depends on the frequency of epileptiform ECoG activity before and after the substance administered and is defined as: frequency value % = (mean of spike frequency after substance administered/mean of spike frequency before substance administered) \times 100

ures 1 C, D). There was no statistically significant difference in the mean frequency and amplitude of epileptiform activity between treadmill exercise groups. The best and earliest decrement was seen in the short-duration treadmill exercise group. The same amount of normal saline (2.5 μ l) did not alter the baseline activity (Figure 1 E).

Discussion

The present study evaluated the effects of short-, moderate- and long-duration treadmill exercise on penicillin-induced epileptiform activity in rats. Although many clinical and experimental studies have shown that epileptiform activity is decreased by exercise, no study has addressed the effects of three exercise formats with short-, moderate- and long-duration treadmill running on penicillin-induced epileptic activity. The mean frequency of epileptiform activity significantly decreased in all treadmill exercise groups. The best and earliest effect was seen in the short-duration treadmill exercise group.

Experimental and clinical studies are important ways to test hypotheses in medicine [23–26]. The penicillin model of epilepsy was used to evaluate the effect of treadmill exercise on epilepsy in the present study, since it bears an electrophysiological resemblance to human focal interictal epileptiform discharges [17].

Exercise has been found to increase cerebral blood flow and amino acid transport across the blood-brain barrier, and alter the release of endogenous peptides and neuronal stimuli [15, 27]. Exercise stimulated both synapses and nerve endings, and prevented age-related degeneration in brain functions [28]. Based on these findings, exercise is anticipated to have favorable effects on epilepsy [29]. The treadmill exercise program (12 m/min speed, 0° inclination and 60% max VO_2) for 10 min/day caused a significant decrease in the rate of pilocarpine-induced epileptic seizures in rats [1, 30]. In a study of 38 people (temporal lobe epilepsy, $n = 17$; control group, $n = 21$) subjected to physical exercise for a minimum of 20 min/day and 3 days per week, none of the participants suffered from seizures during the exercise or at rest, or exhaustion after the exercise [31]. Short-duration bicycle ergometer exercises suppressed epileptic activities and reduced epileptic discharges by preventing EEG changes caused by hyperventilation in 63 young epileptic patients [32]. Eriksen *et al.* [7] performed an exercise program including body building, stretching, aerobics, and dancing for 15 weeks (60 min twice a week) on a total of 15 epileptic women, and noted a significant decrease in the frequency of epileptic seizures at the end of the program. Nakken [6] noted that 63% of 204 epileptic patients were free of epileptic seizures during and after the exercise. Experimental models of epilepsy studies also showed that exercise decreased the intensity of seizure frequency in various models of epilepsy [33–36]. In the study by Arida *et al.* [34] using a pilocarpine-induced epilepsy model (30 min exercise, at a speed of 18 m/min increased to 60 min exercise at a speed of 18–22 m/min; intense aerobic exercise program with 60% VO_2 max), none of the animals demonstrated seizures before or after the maximum oxygen consumption period of the physical exercise. Exercise decreased epileptic seizures and had favorable effects in the prevention of epileptic seizures as a non-pharmacological method [34]. Peixinho-Pena *et al.* [36] applied a 60-day intense exercise program and endurance exercise by using a pilocarpine-induced epilepsy model in rats. The mean frequency of epileptic seizures significantly increased in the control and sham-operated groups after exercise, whereas the frequency of epileptic seizures decreased in training groups compared to the control and sham-operated groups [36]. Swimming exercise for 6 weeks (5 days/60 min) caused a delay in the onset of pentylenetetrazol (PTZ)-induced generalized seizures and significantly decreased the amplitude of PTZ-induced seizures in rats [35]. A swimming and treadmill exercise program for 45 days decreased vulnerability to pilocarpine-induced epilepsy and showed a favorable effect on an ex-

perimentally induced epileptic state [33]. Early-life exercise (starting with 8–10 m/min, increased gradually to 18 m/60 min) reduced epileptic seizures in rats, which might occur in later stages of the life [37]. In line with previous studies, short-, moderate- and long-duration treadmill exercises significantly reduced the mean frequency of penicillin-induced epileptiform activity in rats. The best and earliest effect was observed in the short-duration treadmill exercise group. Moderate- and long-duration swimming exercise did not alter the mean frequency of penicillin-induced epileptiform activity in our previous study [9], although a similar protocol of exercise and a similar epilepsy model were used in the present study, except the type of exercise, compared to the previous study. All types of treadmill exercise caused a decrease in the mean frequency of epileptiform activity. Therefore, it might be suggested that treadmill exercise is more effective than swimming exercise, at least in a penicillin model of epilepsy. However, it was found that short-duration treadmill exercise is more effective in reducing the frequency of epileptic activity in rats than moderate- and long-duration treadmill exercise in the present study. The reason for this is that the physical exercise and type of physical exercise (including duration) may produce different effects on the brain function, since it is well established that physical exercise is able to alter the release of neurotrophins, neurotransmitters, free radicals and neuropeptides in different brain areas which are involved in epilepsy [34, 38–40]. The main limitation of the present study was that all measurements were conducted using only the ECoG recording technique in the epilepsy setting. Further biochemical and neurochemical investigations are required to explore the mechanisms involved in these effects.

In conclusion, the present study indicates that short-, moderate- and long-duration treadmill exercise decreased the mean frequency of penicillin-induced epileptiform activity in rats without changing the amplitude, suggesting a protective role for treadmill exercise in the experimental model of epilepsy. The best and earliest effect appeared in the short-duration treadmill exercise group. The results of the present study suggest that treadmill exercise in all ranges (short, moderate and long duration) is beneficial in reducing the frequency of epileptic activity, at least in the penicillin model of epilepsy. Therefore, recommending appropriate exercise programs and physical activities may help epileptic patients to establish self-confidence and life quality.

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Conflict of interest

The authors declare no conflict of interest.

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