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ORIGINAL ARTICLE

The effects of acute physical activity on severity of seizures induced in rats by homocysteine thiolactone

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Abstract

OBJECTIVES: The aim of this study was to determine the effects of acute, single boost of physical activity on seizures induced by homocysteine thiolactone in rats, using treadmill for small experimental animals as a paradigm of physical activity.

MATERIAL AND METHODS: After adaptation to the treadmill, Wistar albino rats were randomly divided into acute physical activity and sedentary group. Animals from the acute physical activity group ran at the speed of 25 m/min for 30 min, while the sedentary group spent the same time in the treadmill with the speed of 0 m/min. Immediately after completion of the training, all animals were administered with the D, L homocysteine thiolactone at a dose of 5.5 mmol/kg, i.p. Convulsive behavior (incidence, severity, seizure latency and number of seizure episodes per rat) was observed during the next 90 min.

RESULTS: After acute administration of homocysteine, the latency to the first seizure in trained group wasn't significantly shortened, nor the number of convulsive episodes per rat ,compared to the sedentary group of animals. Also, there was no statistically significant difference between groups in the incidence and intensity of convulsive episodes.

CONCLUSION: Based on the results of this study we can conclude that acute boost of physical activity does not potentiates seizures induced by homocysteine thiolactone in rat, what is a favorable conclusion for patients with epilepsy.

Introduction

Regular and individually-tailored physical activity is accompanied by numerous beneficial effects, from the well-known cardioprotective effects, favorable effect on the immune, endocrine and skeletal systems, up to recently reported favorable role in brain disorders (Pimentel *et al* 2015, Hrnčić 2015, The College of Family Physicians of Canada 2010, Stampfer *et al* 2000, Hu *et al* 2001). Properly dosed physical activity could help in prevention of variety of chronic non-communicable diseases, such as hypertension, obesity, depression and others (Cornil *et al* 1965, Wong & Wirrell 2006). Moreover, social integration, personal attitude about own body composition and increased self-confidence are particularly linked with team sport activities (Sperling *et al* 2008, McEwan *et al* 2004). On the other hand, epilepsy, as a very common neurological disorder, is accompanied by a great prejudice and stigma for centuries. Namely, quality of life in these patients is also reduced as a consequence of numerous limitations related to the disease itself and different comorbidities. Patients with epilepsy were discouraged to take part in physical activity and team sport due to their and their physicians fear of seizure induction or incensement in their frequency (Fountain & May 2003); what resulted in high rate of social isolation of children as well as the adolescents with this disease (McEwan *et al* 2004). Unfortunately, even nowadays when our opinions on the role of physical activity has been changed, patients with epilepsy are still far less active than the general population (Arida *et al* 2008). When it comes relation between physical activity and epilepsy, researchers still have. Somewhat contradictory results on the relationship between physical activity and epilepsy could be find in the literature: some emphasizing protective role of exercise (Arida *et al* 2004, Souza *et al* 2009), others pointing out proepileptiform (i.e. proicatal) effects (Kuijer 1980, Steinhoff *et al* 1996), particularly of on the role of acute, single-boosts of physical activity, contrary to recently partially revealed chronic, sustained forms (Arida *et al* 2008, Hrncic 2014, 2015).

Experimental models of seizures are of particular value for assessment of different mechanisims underlying brain hyperexcitability, as well modalities of its modulation. Among them, seizures induced in rats by homocysteine thiolactone represents model of generalized epileptic activity. Namely, homocysteine, a sulfurous amino acid, is formed by methionine metabolism (Hoffer 2005). It is involved in crucial biological processes and homocysteine thiolactone has been considered as its most reactive metabolite. Elevated level of homocysteine is established risk factor for cardiovascular disease, various cancers, as well as numerous brain disorders (Sachdev 2005, Kado *et al* 2005). Stanojlović *et al* (2009) showed that acute administration of homocysteine thiolactone to adult rats, significantly alter neural circuits, with consecutive seizures in animals' behavior.

The aim of this study was to determine the effects of acute, single boost of physical activity on seizures induced by homocysteine thiolactone in rats, using treadmill for small experimental animals as a paradigm of physical activity.

Materials and methods

Experiments were performed on adult males of Wistar rats (230–260 g), obtained from the Military Medical Breeding Laboratories, Belgrade, as a local accredited supplier. Animals were kept under controlled laboratory conditions (ambient temperature 21–23 °C, 55–65% humidity, 12/12 light/dark cycle, with light starting on 8 a.m.) and had free access to standard laboratory chow and tap water.

All experimental procedures were in full compliance with Directive of the European Parliament and of the Council (2010/63/EU), as well as with ethical standards in sport and exercise science research. The Ethical Board of Belgrade University Faculty of Medicine and The Ethical Committee of the Republic of Serbia (Permission No 298/5-2) approved these experiments.

Treadmill apparatus (NeuroSciLaBG-Treadmill, Elunit, Serbia) for small experimental animals was used in the study (Hrnčić *et al* 2012). Adaptation of animals to the apparatus and running conditions done in 3 daily sessions of 10 min with belt speed increasing from 5 to 10 m/min. After this adaptation, rats were randomly divided into 1) acute physical activity and 2) sedentary group. Animals from the acute physical activity group ran at the speed of 25 m/min for 30 min (n=8), while the sedentary group spent the same time in the treadmill with the speed of 0 m/min (n=10). Immediately after completion of the assigned treadmill protocol, all animals were administered with the D,L homocysteine thiolactone at a subconvulsive dose of 5.5 mmol/kg, i.p. Convulsive behavior was observed during the next 90 min, as previously reported (Stanojlovič *et al* 2009). Briefly, convulsive behavior was assessed by the incidence of motor seizures, seizure latency, number of seizure episodes per rat and their severity. Seizure episodes severity was determined by a modified descriptive – rating scale as with grades defined as follow: 1 – head nodding, lower jaw twitching; 2 – myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); 3 – progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail; 4 – prolonged severe tonic-clonic convulsions lasting over 20s (status epilepticus) or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min).

Incidence of seizures and lethality data were evaluated by Fisher's exact probability test. Normal distribution of the data on seizure latency, number and severity of seizure episodes, as well as number and duration of SWD has not been estimated by Kolmogorov – Smirnov test. Therefore, the non-parametric analyses (Kruskal-Wallis ANOVA and Mann-Whitney U-test) were used to analyze these data. Medians with 25th and 75th percentiles were used to present these results.

Results

Convulsive behavior of animals from acute physical activity group, upon administration of homocysteine thiolactone was characterized by incidence of 37%. The seizure incidence was not statistically different between acute trained and sedentary animal groups ($p>0.05$; Figure 1).

The latency period to the first seizure was not significantly prolonged in acute physical activity group in comparison to the sedentary group of animals ($p>0.05$; Figure 2A).

Number of convulsive episodes per animal in the group underwent acute physical activity actvity was not stistically significant difference to sedentary group ($p>0.05$; Figure 2B).

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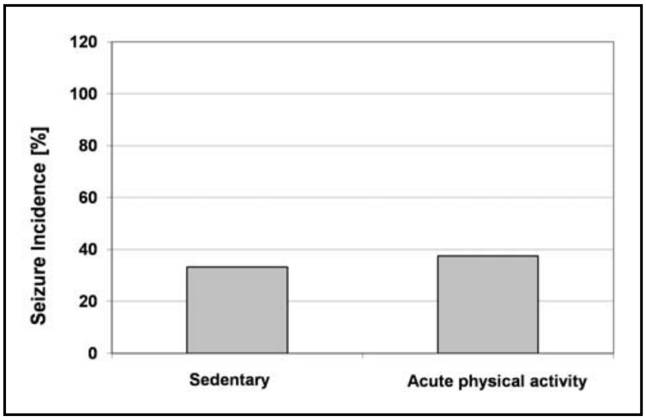

Figure 1. The effect of acute physical activity on the incidence of seizures induced by homocysteine thiolactone. After adaptation on treadmill, rats were randomly divided into acute physical activity (treadmill run at the speed of 25 m/min for 30 min, n=8), and sedentary group (the same time in the treadmill with the belt speed of 0 m/min, n=10). Immediately after completion of the assigned treadmill protocol, all animals were administered with the D,L homocysteine thiolactone at a subconvulsive dose of 5.5 mmol/kg, i.p. Incidence: the percentage of animals seizuring in comparison to the total number of animals in the group. Statistical significance of differences in the seizure incidence was assessed by Fisher's exact probability test ($p>0.05$).

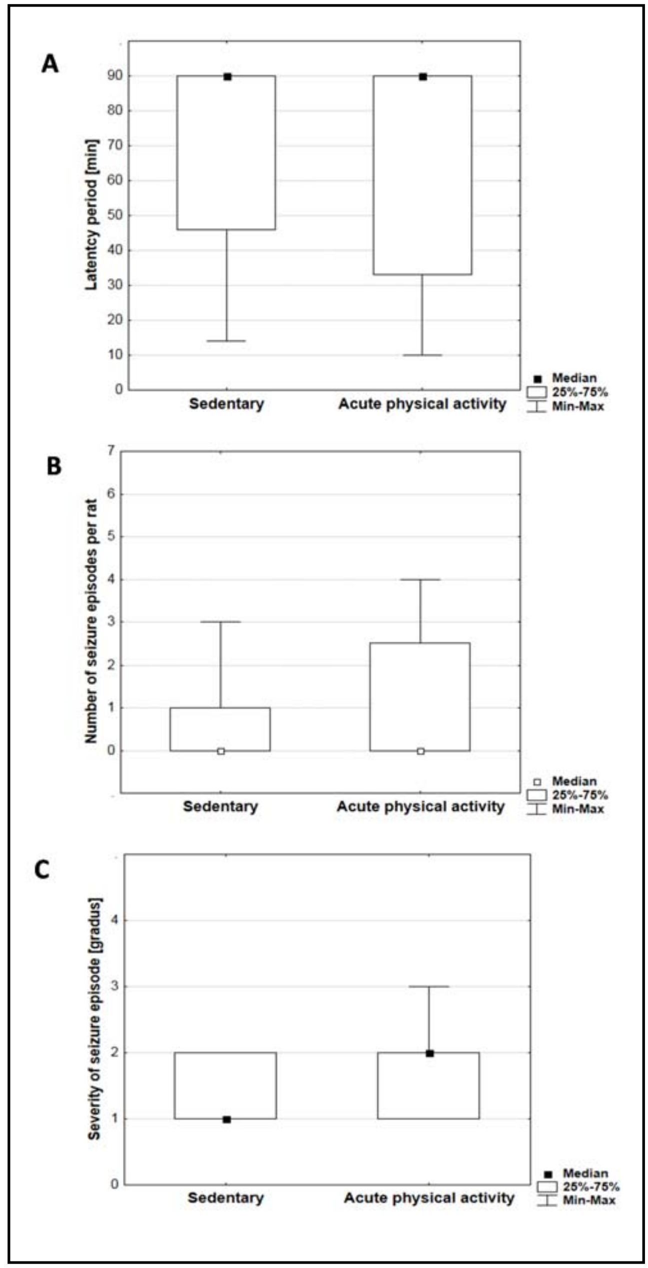

Figure 2. The effect of acute physical activity on the latency period (**A**), number of seizure episodes per rat (**B**) and their severity (**C**) in model of homocysteine thiolactone-induced seizures. Latency period is defined as a time from administration of homocysteine thiolactone to the development of first seizure response. Seizure severity was evaluated by descriptive rating scale with grades from 0 to 4. The statistical significance of the differences between groups was determined by Mann-Whitney U test ($p>0.05$). For details see caption to Figure 1.

In acute physical activity group, the median of the seizure intensity was of the grade 2, while in the sedentary group was of the grade 1, but this difference didn't attain statistical significance ($p>0.05$; Figure 2C).

Discussion

The results of this study indicated that the acute physical activity in rats did not significantly affected any parameter of convulsive behavior, which further means that acute physical activity does not have proexcitatory role in this seizure model. Although there has been a mild change to the incidence, as well as in the number of seizures between the experimental and control group, there were not statistically significant differences. These results are in line with studies showing unaltered or decreased seizure frequency in exercised patients (Eriksen *et al* 1994, Nakken *et al* 1990). This is also confirmed in a study where patients EEG were registered during the exercise (Arida *et al* 2010).

In this study we used running on a treadmill as a paradigm of physical activity, a method used and favored in numerous studies (Arida *et al* 2007). However, beside treadmill, swimming is also used as a model of physical activity (Souza *et al* 2009, Tutkun *et al* 2010, Lai & Trimble 1997) and both have their advantages and disadvantages related to ability to adjust the workout level, level of stress and type of behavior (acquired or inborn). In treadmill method, workout effort level is adjustable more easily, while swimming is inborn behavior (discussed in more details in Hrncic 2015). Indeed, experimental studies using swimming as a form of physical activity showed results that are congruent with the results obtained by running on the treadmill.

In certain situations, acute boost of physical exercise could be accompanied by side effects such as stress and hypoxemia which can potentiate seizures (LaPlente 2010). The potential role of these factors has been proven also in clinical settings (Spector *et al* 2000, Spatt *et al* 1998). Also, it has been proven that stress causes structural changes in certain parts of the brain closely associated with hyperexcitability (Magarinos *et al* 1997).

Individually set dose of physical activity is very important for distinguishing its effects, since exceeding the physical body limits can lead to serious consequences. For instance, jogging tempo which is comfortable for one persons, in other can cause profuse sweating and cardiac arrhythmias, which consequently cause hypoxia (Möhlenkamp *et al* 2008, Benito *et al* 2011). This is the limit when exercise becomes intense and varies from person to person. For patients with epilepsy evaluation of these limits may be of critical importance (Krivoshchekov & Lushnikov 2011). Sudden onset of exercise, after a period of physical inactivity, may also adversely affect the control of the seizures.

Taking into consideration the results that we got, and previous research in this field, we can assume that acute physical activity will not potentiate the seizure, as long as it falls within the limits of physiological body capabilities and capacity. This is of particular importance regarding antiepileptic therapy and levels of plasma homocysteine. Sedentary life style in these patients may be an additional risk factor for seizures, having in mind that hyperhomocysteinemia has been shown to be diminishable by physical activity (Okura *et al* 2006, Hrncic *et al* 2014). Sedentary life style in these patients may be an additional risk factor for seizures, having in mind that hyperhomocysteinemia has been shown to be diminishable by physical activity (Okura *et al* 2006).

Based on the results of this study we can conclude that acute physical activity does not potentiates seizures induced by homocysteine in rats. The role of physical activity in epilepsy is complex. We believe that recent studies in this filed will induce transitions in patient's estimation of physical activity and that many positive effects of physical activity ranging from cardioprotective role, psychosocial integration to the promising anticonvulsive effects will sustained.

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