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EFFECT OF ANAEROBIC AND AEROBIC EXERCISE TOWARD SEROTONIN IN RAT BRAIN TISSUE

SIÇAN BEYİN DOKUSUNDAKİ SEROTONİNE YÖNELİK OKSİJENLİ VE OKSİJENSİZ ÇALIŞMA ETKİSİ

Rostika Flora*[1], Theodorus[2], Muhammad Zulkarnain[2], Rahmad Aswin Juliansyah[3], Syokumawena[3]

Abstract

Physical exercise plays a substantial role in maintaining our heath. In the molecular level, physical exercise induces the release of neurotransmitter, such as serotonin. Lack of serotonin could lead to stress or depression condition. We assumed that physical exercise could increase serotonin level in the brain. Therefore, this study aimed to investigate the effect of anaerobic and aerobic exercise toward serotonin level in male Wistar rat brain tissue. Twenty-eight male Wistar rats were divided into seven groups consist of control; 1x, 3x, 7x of aerobic exercise; and 1x, 3x, 7x of anaerobic exercise which conducted in a week. A rat treadmill was used at speed 35 m/min during 20 min for anaerobic exercise, and it was used at speed 20 m/min during 30 min for aerobic exercise. Serotonin level was measured using ST/5-HT (Serotonin/5-Hydroxytryptamine) ELISA Kit. Significant differences between treatments were tested by ANOVA (α = 5%). In contrast, both of anaerobic and aerobic exercise had lower serotonin level than the control.

Keywords: serotonin/5-hydroxytryptamine, anaerobic, anaerobic, physical exercise

Özet

Fiziksel egzersiz sağlığımızı korumamızda hayati bir rol oynar. Moleküler seviyede fiziksel egzersiz serotonin gibi sinir iletici lerinin salgılanmasına neden olur. Serotonin eksikliği stres ya da depresyon durumuna sebep olabilir. Fiziksel egzersizin beyindeki serotonin seviyesini artırdığını varsaydık. Bu nedenle, bu çalışmada erkek Wistar sıçanları beyin dokusundaki serotonine yönelik oksijenli ve oksijensiz çalışma etkisini araştırmayı amaçladık. 28 adet erkek Wistar sıçanı, bir haftada gerçekleştirilen 1x, 3x, 7x'li oksijenli ve oksijensiz çalışma grupları olmak üzere 7 adet kontrol grubuna ayrılmıştır. Sıçan çarkı, oksijensiz çalışma için 20 dakika boyunca 35 m/min hızda kullanılırken oksijenli çalışma için 30 dakika boyunca 20 m/min hızda kullanılmıştır. Serotonin seviyesi ST/5-HT (Serotonin/5- Hidroksitriptamin) ELISA Kit kullanılarak ölçülmüştür. Tedaviler arasında önemli farklılıklar ANOVA (α = 5%) ile test edilmiştir. Varsayımımızın aksine, hem oksijenli ve hem oksijensiz çalışma gruplarının kontrol grubundan daha düşük bir serotonin seviyesine sahip olduğu ortaya çıktı.

Anahtar Kelimeler: *Serotonin/5- Hidroksitriptamin, oksijenli, oksijensiz, fiziksel egzersiz*

*[1]Corresponding author: Study Program Biomedical Science, Faculty of Medicine, University of Sriwijaya, Palembang, Indonesia.
E-mail: rostikaflora@gmail.com
[2]Faculty of Medicine, University of Sriwijaya
[3]Postgraduate Student of Biomedical Science, Faculty of Medicine, University of Sriwijaya

1.Introduction

Lack of physical activity leads to increase many health problems. The World Health Organization noted that lack of physical activity is the fourth death factor (6% of deaths worldwide) (WHO, 2010). Based on its metabolism, there are two types of physical activity, aerobic and anaerobic. Aerobic exercise is a physical activity that uses ATP from the oxidative phosphorylation of glycogen and free fatty acids which depends on the availability of oxygen. In contrast, anaerobic exercise uses ATP from glycolysis which no need of oxygen (Astrand et al., 2003).

Physical activity could induce the release of serotonin (Klempin et al., 2013) (Lin & Kuo, 2013) (Meeusen & De Meirleir, 1995). Serotonin (5-hydroxytryptamine), a monoamine scattered in the human body, acts as a neurotransmitter in the synapse of nerve cells (Charnay & Leger, 2010) (Pytliak et al., 2011). Serotonin has substantial roles in the physiological function of the human body, including thermoregulation, regulation of cardiovascular, locomotion, pain, reproduction, sleep-wake cycle, memory, cognition, aggression, responses to stressors, emotions and mood (Charnay & Léger, 2010). Serotonin dysfunction leads to illness, such as depression, schizophrenia, anxiety and panic, migraine, hypertension, pulmonary hypertension, eating disorders, vomiting and irritable stomach syndrome (Pytliak et al., 2011).

According to Lin and Kuo (2013), an increase in synthesis and secretion of serotonin in the serum and in the central nervous system is affected by the intensity of physical activity. Physical exercise by increasing serotonin can affect emotional improvement in patients with major depression (Ahmad et al., 2007). Research conducted by Chaouloff et al., (1985) showed that Wistar rats treated on a treadmill for 60 and 120 minutes, 20 m/min, during 4-5 times exercise in a week revealed an increase of serotonin level. Physical exercise could improve the cognitive function and mental health. Light physical exercise raises the neuronal activity of hippocampus by increasing the neurotrophic factor and neurogenesis factor (Okamoto & Soya, 2012). The study conducted by Wang et al., (2013) showed an increase of serotonin level in the Wistar rat hippocampus after treated in the running wheels for 2 km in 4 weeks. The hypothesis was that both exercises (anaerobic and aerobic) could increase the serotonin level in the rat brain.

Study about the effect of frequency of the exercise toward the serotonin was limited. Prior study in rats revealed that intense frequency of anaerobic and aerobic exercise (seven times in a week) generated a heart infraction, while once and three times exercise in a week were safe (Flora et al., 2012). That indicates that intense physical exercise, such as seven times in a week, damage the heart muscles. However, the effect of the exercise in moderate or intense frequency toward serotonin level in brain tissue was unknown. Therefore, this study aimed to observe the effect of once, three, and seven times anaerobic and aerobic exercise in a week toward serotonin level in Wistar rat brain.

2.Material and Method

2.1.Experimental Design

This study was in vivo experimental study in posttest-only control design (Brink et al., 2005). This study was conducted in February 2015 at animal house laborator, Pharmacology and Therapy, Faculty of Medicine, Padjajaran University, Bandung. This study was approved by the Ethic Committee of the Faculty of Medicine Universitas Sriwijaya and Ethic Committee of the Mohammad Hoesin General Hospital Centre, Palembang, Indonesia.

2.2.Animal Preparation

Twenty-eight healthy and adult male rats, 6-8 weeks old, 140-250 g (*Rattus norvegicus* strain Wistar) were used. The amount of sample was determined using Federer's formula (Federer, 1991): $(n-1)\ (t-1) \geq 15$

The seven groups of treatment were conducted ($t = 7$) including control, 1x, 3x and 7x exercise in a week of anaerobic and aerobic treatment and each group was consist of four healthy and adult male rats ($n = 4$). All rats were obtained from Institut Teknologi Bandung, Bandung, Indonesia.

2.3.Treatment

Treatments were divided into control with no exercises, anaerobic exercise and aerobic exercise. A rat treadmill was used for both of anaerobic and aerobic treatment. The frequencies of anaerobic and aerobic exercise were the same. Both of anaerobic and aerobic exercise were conducted in once (1x), three times (3x), and seven times (7x) in a week (Flora et al, 2012). However, aerobic exercise was set at speed 20 m/min for 30 min, while anaerobic exercise was set at speed 35 m/min for 20 min (Soya et al., 2007; Fahrenia et al., 2009, and Flora et al., 2012). Acclimatization of lab condition was conducted in a week for all groups while treadmill acclimatization was conducted for the anaerobic and aerobic group (Kregel, 2006). All rats body weight was measured before and after the treatment.

2.4.Brain Preparation

Rat brain tissues were cleaned with PBS (0.01 M, pH = 7.4), then all of them were homogenized by MagNa Lyser Green Beads (5.000 x g, 60 seconds). All samples were centrifuged at 5.000 x g for 5

minutes. Then, the supernatant was collected and stored at -70oC.

2.5. Serotonin Measurement

50 µL supernatant of each sample was used for Serotonin assay. Serotonin level was measured based on competitive ELISA (Enzyme-linked Immunosorbent Assay) using ST/5-HT (Serotonin/5-Hydroxytryptamine) ELISA kit & its protocol (E-EL-0033, Elabscience).

2.6. Statistical Analysis

The data were analyzed using SPSS 19 for windows and were presented as means ± standard error. Analysis of variance (ANOVA) followed by Tukey HSD post-Hoc test was used to compare mean of serotonin level from the control, anaerobic, and aerobic exercise. Independent T-test was used to compare the mean of serotonin level between each frequency in aerobic and anaerobic exercise. α = 0.05 was considered as the significant difference level.

3. Result

Measurement data of rat weight, before and after treatment, was conducted to observe the effect of aerobic and anaerobic exercise to the rat weight. The result showed that anaerobic exercise revealed a higher reduction of rat weight (1.5 – 2.75 g) than the aerobic exercise.

Serotonin level of the aerobic and anaerobic group was lower than serotonin level of the control. The lowest serotonin was observed in 1x aerobic exercise (0.006 ±0.003 ng/ml) and the highest was observed in the control (0.709±0.063 ng/ml). ANOVA showed an insignificant difference between 1x, 3x, and 7x of anaerobic or aerobic exercise (Table 1). Independent t-test showed an insignificant difference in 3x (p=0.5) and 7x (p=0.151) of the anaerobic and the aerobic exercise, whereas a significant difference (p=0.000) only occurred between anaerobic and anaerobic exercise in 1x exercise (Table 1).

Table 1: The Serotonin level is numbers before (left side) "±" symbol, all numbers after (right side) "±" are the standard error in male Wistar rat brain tissue from all treatments

Treatment Group	Level (ng/ml)	
	Anaerobic	Aerobic
Control	0.709±0.063 a	0.709±0.063 a
1x	0.074±0.004 bA	0.006±0.003 bB
3x	0.084±0.003 bA	0.065±0.025 bA
7x	0.099±0.034 bA	0.034±0.007 bA

Note. Data each group (n=4) were presented as mean ± standard error. Different lower case letters (a,b) in the same column show a significant difference between control, 1x, 3x, and 7x treatment (p<0.05, Tukey HSD post hoc test). Difference upper case letters (A,B) in the same row show a significant difference between the anaerobic and the anaerobic exercise in each frequency (p<0.05, t-test 2-tailed).

4. Discussion

Serotonin, a derivative of tryptophan, has substantial roles in human physiology function, such as thermo regulator, nutrition control, cardiovascular, reproduction function, pain, aggressiveness, sleeping cycle, memories, cognition, mood, emotion and stress response (Charnay & Leger, 2010). Regular and continuous physical exercise could increase the serotonin level by relaxing the body and increasing the serotonin expression and secretion in serum or in central neuron system (Hassan & Amin, 2011) (Lin & Kuo, 2013). Study result of observing human as object showed that 3 times Pilates exercise during 12 weeks increased the serotonin level and reduced the stress (Hassan & Amin, 2011). Particular time, approximately 8 to 12 weeks after continuous exercise or training is needed by the body to be adapted and make any positive effects (Willmore & Costill, 1999; Astrand et al., 2003). The prior study revealed that physical exercise would give positive effect after 10 weeks of training (Fiatarone, et al, 1994). Otherwise, Wilson and Marsden (1996) research showed that serotonin level increased in rat's ventral hippocampus after the rats were treated with 60 minutes treadmill exercise for 20 m/min/day during 4-5 weeks. Another research showed that serotonin level increased when treated animals were treated in aerobic exercise for 3 times a week during 20 weeks (Valim et al., 2013).

A significant difference of serotonin level between 1x aerobic and 1x anaerobic exercise group was observed. Among aerobic and anaerobic treatment the lowest serotonin level was observed at 1x aerobic exercise (0.006±0.003 ng/ml), and the highest serotonin level was observed at the control followed by the 7x anaerobic exercise. However, this study showed that the exercises done just in a week had lower serotonin level than serotonin level in the control. According to the result, the short duration of exercise did not induce the increase of serotonin secretion. One week of treatment made the given exercises presented early as a stressor rather than as a stimulus to increase serotonin level in brain tissue. Physical exercise done intensively without rest is an acute exercise could induce a stress to the body and decrease the serotonin level in the brain. The study by Chen et al., (2008) showed that Sprague–Dawley rat treated by running 9 m/minute for 60 minutes every day during four weeks revealed a decrease of serotonin level in its hippocampus.

The main limitation of this study was the duration of the treatment. One week of exercise was not long enough to see a positive effect of the exercises. We suggest for measuring the serotonin in a specific place of serotonin (hippocampus) rather than in whole brain tissue. Moreover, duration of the treatment and histopathology of the brain tissue should be prolonged and observed to know clearly about the effect of anaerobic and aerobic

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exercises toward the serotonin level. In conclusion, both of physical exercise done during a week in three different frequencies could not increase the serotonin level in rat brain. Besides, a short time of anaerobic and aerobic exercise led to decrease the serotonin level in rat brain.

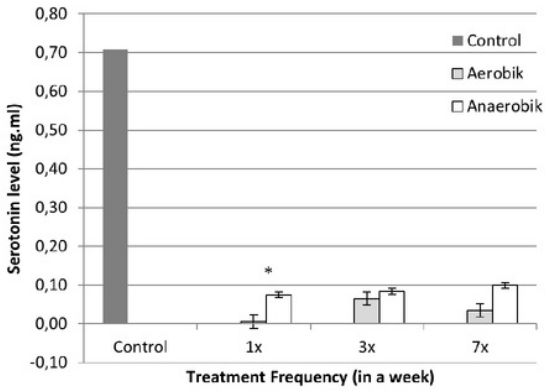

Fig. 1: Column chart of serotonin level in control, aerobic and anaerobic treatment. The (*) shows a significant different of serotonin level between 1x anaerobic and 1x aerobic treatment.

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