

Intensity Of Swimming Exercise As Chronic Stress Induction Towards Expression Of Glucose Transporter 1 (Glut1) In Brain Capillary Endothelial Of Rats (*Rattus Norvegicus*)

by Lilik Herawati

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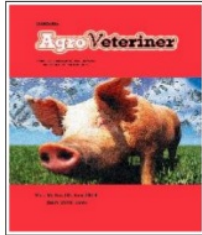
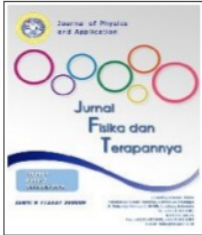
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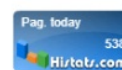
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INTENSITY OF SWIMMING EXERCISE AS CHRONIC STRESS INDUCTION TOWARDS EXPRESSION OF GLUCOSE TRANSPORTER 1 (GLUT1) IN BRAIN CAPILLARY ENDOTHELIAL OF RATS (*Rattus norvegicus*)

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ABSTRAK

Stres dapat menyebabkan efek positif atau negatif pada tubuh, tergantung pada durasi dan intensitas stres. Stres berkepanjangan akan menyebabkan penyakit neurodegeneratif atau gangguan kognitif melalui gangguan dalam sistem neurotransmitter otak, tingkat biomolekuler otak, dan metabolisme otak. Regulasi potensial Glucose Transporter-1 (GLUT1) dalam respon sawar darah-otak terhadap berbagai kondisi patologis otak yang berhubungan dengan stres. Tujuan penelitian ini adalah untuk menjelaskan pengaruh intensitas stres kronis terhadap ekspresi transporter-1 glukosa pada otak tikus. Penelitian ini menggunakan 30 ekor tikus (*Rattus norvegicus*) galur wistar jantan yang dibagi menjadi 3 kelompok: kelompok kontrol (K0), perlakuan latihan berenang dalam intensitas berat (K1) dan latihan berenang dalam intensitas ringan (K2). Pemeriksaan dilakukan terhadap ekspresi GLUT1 pada sel endotel otak yang diamati menggunakan teknik pewarnaan imunohistokimia. Hasil penelitian menunjukkan bahwa ada penurunan ekspresi GLUT1 di endotel otak akibat latihan renang intensitas berat dan latihan renang intensitas ringan. Depleksi ekspresi GLUT1 dalam endotel otak kelompok intensitas berat lebih besar dari pada kelompok intensitas ringan dengan $p < 0,05$. (FMI 2012;48:24-27)

Kata kunci: GLUT1 (glucose transporter-1), stres kronik, otak

ABSTRACT

Stress can lead positive or negative effect on the body, depending on the duration and intensity of stress. Prolonged stress will cause neurodegenerative disease or cognitive impairment through disruption in brain neurotransmitter systems, biomolecular level of the brain, and brain metabolism. Potential Regulation of Glucose Transporter-1 (GLUT1) in the blood-brain barrier responds to various stress-related brain pathological conditions. The purpose of this study is to explain the influence of chronic stress intensity towards expression of glucose transporter-1 in the brains of rat. This study used 30 rats (*Rattus norvegicus*) male wistar strain which divided into 3 groups: control group (K0), the treatment of swimming training in severe intensity (K1) and swimming training in mild intensity (K2). Examination was conducted on GLUT1 expression in endothelial cells of the brain that be observed using immunohistochemical staining techniques. The results showed that there was depletion of GLUT1 expression in brain endothelial due to swimming training in severe intensity and swimming training in mild intensity. GLUT1 expression depletion in brain endothelial of severe intensity group was greater than in mild intensity group with $p < 0.05$. (FMI 2012;48:24-27)

Keywords: GLUT1 (glucose transporter-1), chronic stress, brain

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INTRODUCTION

Stress has become a part of human and other organisms' life that can cause effects of on change in the organism, both physiological and psychological. Stress, as first proposed by Hans Selye, stress physiologist in 1976, is the body's non-specific response to various stressful or threatening situation to the organism. Prolonged stress, besides being recognized as the aetiopathogenesis of several metabolic diseases such as hypertension, coronary heart disease, and diabetes, can also cause changes in brain function (Savtchouk & Liu 2011). The

brain as one of vital organs of the body is an organ that is highly dependent on glucose as an energy provider and for the synthesis of essential amino acids as brain neurotransmitter. If the glucose levels in the brain is in a state of homeostasis and uncompensated, brain function can be impaired (Benton et al 1996).

The presence of glucose transporter proteins (GLUT) in the brain is important for ensuring glucose supply to the brain. To date, three GLUT isoforms have been identified to have significant role in brain, i.e. GLUT1, GLUT3, and GLUT5. GLUT1 has the highest

concentration in the brain capillary blood vessels endothelium. Although the capillary blood vessels of the brain is only about 2% of total brain volume, the presence of GLUT1 in these capillaries remain the focus of several studies because of GLUT1 is glucose transporter that has to be passed by glucose to enter the brain (Maher et al 1994).

GLUT1 is GLUT whose expression is induced by cellular stress state (stress-inducible proteins) (Wertheimer et al 1991). GLUT1 expression can be increased when cells are stressed (Carruthers et al 2009, Barros et al 1997). However, prolonged stress causes impaired synthesis of GLUT1 so that GLUT1 expression decreases (Duelli & Kuschinsky 2001). Physical exercise, in addition to having benefits for the body, is one of the stressors that can cause physiological changes in the body. Swimming as a form of physical exercise is a physical stressor easily used to model the stress in experimental animals. Swimming is not just a simple exercise, but the work load in swimming is high and the involvement of emotional factors in animals is difficult to eliminate that are likely to lead to a more integral stress response (Nayanatara et al 2005, Bhatia et al 2011). Level of stress such as differences in the intensity of swimming causes different physiological responses to cellular level. However, the effect of physical exercise as stress induction on the expression of GLUT1 was not yet clear. Based on the description above, the authors wanted to determine the effect of prolonged stress by inducing stress through physical exercise of swimming in different degrees of intensity on decreased expression of glucose transporter-1/GLUT1 in rats' brain.

MATERIALS AND METHODS

The subjects were male Wistar rats aged 2-3 months, weighing 150-160 grams. Sample size was 30 rats. This study used a randomized post test only control group design. Eligible rats were divided into 3 groups: control group or no treatment (K0), the treatment group with high intensity swimming exercise (K1), and the treatment group with low intensity training exercise (K2). Physical stress used to was swimming exercise to induce stress (Forced Swimming Induced Stress) by modification of the method developed by Porsolt et al in 1977 (cited by Nayanatara et al 2005).

Swimming exercises for stress induction was done by making the animals, which had been given with a load 3% of the body weight, to swim for 70% of swimming maximum capacity for treatment group K1 and 30% of

swimming maximum capacity for treatment group K2, or until the animals experienced fatigue during the session of stress provision. The animals swam in a place that had been filled with water in a temperature of $25 \pm 2^\circ\text{C}$ and a water depth of 30 cm. Swimming exercise was performed one session per day for 7 consecutive days for the effects of chronic stress (Nayanatara et al 2005). Experimental animals were sacrificed on the last day of the treatment, the seventh day, at the same time for all control and treatment groups, then we performed animal brains harvesting.

Preparation of brain tissue was made by the method of paraffinization. Then, GLUT1 expression was characterized using immunohistochemical staining. The counting of GLUT1 expressing capillary endothelium was performed quantitatively express in visual field as many as the average number of brain capillaries cut transversely on a piece of brain tissue slides.

RESULTS

The study lasted for 3 months with no samples of experimental animals died or dropped out as the research proceeds. Preparation, immunohistochemical staining, and observation of GLUT1 expression in brain tissue could be performed on the entire sample. GLUT1 expression profile in brain capillary endothelium by immunohistochemical staining in control group and treatment group can be seen in Figure 1.

GLUT1 expression assessment was performed in 18 brain capillaries cut transversely, which was about the average capillary number of whole sample preparations. The descriptive analysis of GLUT1 expression in brain endothelial shows that there was a decrease in GLUT1 expression in both treatment groups compared to that in control group. The least GLUT1 expression was present in treatment group with high intensity swimming exercise followed treatment group with low intensity swimming exercise.

The results of analytical analysis using ANOVA showed significant differences ($p < 0.05$) of GLUT1 expression in brain capillary endothelium between groups with $p = 0.0001$. Least significance differences between groups was followed by Least Significance Difference (LSD) test which also showed significant values ($p < 0.05$) with $p = 0.0001$ for the difference between K0 and K1, $p = 0.03$ for the difference between K0 and K2, and $p = 0.03$ for the difference between K1 and K2.

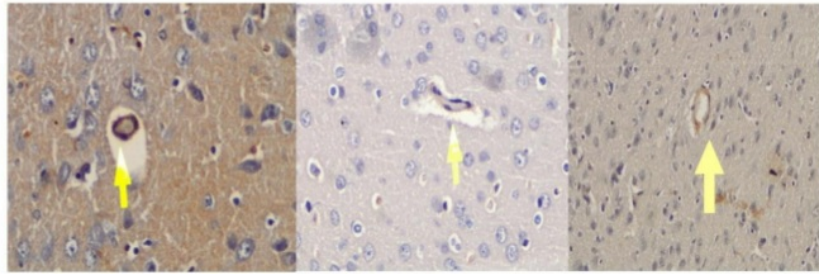


Figure 1. GLUT1 expression profile in brain capillary endothelium in control group (K0), treatment group with high intensity swimming exercise (K1), and treatment group with low intensity swimming exercise (K2)

DISCUSSION

Stress induced by swimming exercise (Forced Swimming Induced Stress) is a type of physical stress effectively used to study the physiological changes and the capacity of organism to respond to stress using experimental animals. This is because swimming exercise is not just a simple exercise, but it also involves emotional factors that influence the degree of stress in experimental animals (Nayanatara et al 2005). Physiological stress is needed to activate various responses of the body as a defense mechanism. This is the expected positive effects of stress, the stress that can be tolerated by the body. Prolonged (chronic) stress or with excessive intensity can cause pathological negative effects on the body because the body experiences fatigue, resulting in organ dysfunction and even death (Selye 1950). The difference in chronic stress exposure intensity to an organism, such as low or high intensity, may cause different organism's response to stress.

The results showed that there is a decrease in brain endothelial GLUT1 expression in treatment group with high intensity swimming exercise (K1) with a mean of 28.20 ± 6.60 and treatment group with low intensity swimming exercise (K2) with a mean of 35.90 ± 9.39 when compared with the control group (K0) which had a mean of 43.60 ± 6.04 . The results of the ANOVA analysis showed that there were significant differences in GLUT1 expression data on brain endothelium. Results of LSD between each group showed significant decrease with $p < 0.05$. This suggests that swimming exercise as chronic stress induction can cause changes in GLUT1 expression in brain endothelium.

Decrease in GLUT1 expression in brain endothelial due either to high or low swimming exercise intensity in this study was in accordance with some literature indicating that chronic or repeated stress is one mechanism that

causes neurodegeneration through hypersecretion of glucocorticoids. Neurodegeneration can lower mRNA levels of a protein, including membrane transporters, through the inhibition of insulin secretion, an important hormone in protein synthesis (Somwar et al, 1998, Munhoz et al 2008).

GLUT1 expression in brain endothelial of treatment group with high intensity swimming exercise showed higher decrease than that in treatment group with low intensity swimming exercise with a significance level of $p = 0.03$. It resulted from damage of endothelial membranes due to excessive stress intensity, coupled with higher decrease in GLUT1 mRNA synthesis in the treatment group with high intensity swimming exercise than that in group with low intensity swimming exercise, so that the cell membrane GLUT1 expression would also decrease even though it received adequate activation signals (Somwar et al, 1998, Munhoz et al 2008).

Physical stress is a trigger of oxidative stress that may increase the levels of free radicals in the body. Chronic stress with tolerable intensity may reduce level of cell oxidative stress and improves cell ascorbic acid as an endogenous antioxidant in the brain. This provides the beneficial effects of physical exercise (Liu et al 2000). Other literature about the effect of free radicals on protein synthesis mentioned that excessive free radicals formed will increase oxidative damage, inhibit insulin gene expression, decrease insulin secretion, and ultimately lower protein synthesis (Mulukutla et al 2010, Somwar et al 2000). The intensity of chronic stress in experimental animals in this study was actually a submaximal and light intensity. However, the emotional factor that also involved in the experimental animals added the stress intensity so that the pathological effects of the stress became predominant, indicating by the decrease in GLUT1 expression in

brain endothelial and glial cells of the experimental animals.

The decrease in GLUT1 expression in rat brain capillary endothelium, both in high and low intensity swimming exercise treatment as chronic stress induction, indicated that difference in the intensity or degree of stress lowers brain GLUT1 expression. Chronic stress given continuously or without time lag causes the body does not have time for adaptation and recovery process as a form of compensation to overcome the damage up to cellular level that occurs as a result of stress. Long-term and continuous stress treatment induces cell malfunctions.

CONCLUSION

High intensity swimming exercise as chronic stress induction causes the highest decrease in capillary endothelial GLUT1 expression of white rat's brain. Low intensity swimming exercise as chronic stress induction also causes a decrease in capillary endothelial GLUT1 expression of white rat's brain with a statistically significant reduction compared to that in untreated control group. Further research is needed to determine the levels of biological stress markers in experimental animals to find the proper dosage of stress for experimental animals.

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