Relationship of Adrenaline Administration to Increased Blood Sugar Levels in Male Rattus Norvegicus Rats

Abstract

Stress is a condition that causes a steady state in the body to be disrupted. When a stressor hits the body, the hypothalamus will send a signal to the adrenal gland to produce catecholamine. One of the catecholamines is adrenaline. Adrenaline will increase heart muscle contraction, dilatation of blood vessels, and increase glycogenolysis and gluconeogenesis, increasing blood glucose. This experimental research with research subjects is male *Rattus norvegicus*, 24 weeks, 250-300 gr, and healthy. The total sample is 24 rats divided into four groups, with two groups of controls not injected with adrenaline and two groups of treatment. Each treatment group will be differentiated between fasting groups (P1) and not fasting groups (P2) and injected with adrenaline 0,6 mg/kgBB via intraperitoneal. This research uses pre-test and post-test methods to measure blood glucose levels. The one-way ANOVA result shows a significant difference ($p<0,05$) between the pre-test and post-test of blood glucose level.

Keywords: Adrenaline, Blood Glucose, Stress.

Introduction

Adrenaline is a hormone secreted by the adrenal glands in the medulla. [1] The adrenal medulla, which makes up 28% of the adrenal gland, is formed by granular cells that are intensively innervated by a cell-filled pre-ganglionic neural network. The two cell types can be distinguished morphologically. Adrenaline-secreting cell types have large, sharp granules, and noradrenaline-secreting cell types have smaller, blunt granules that do not fill all the space. In the human body, 90% of cells are the adrenaline-secreting type, and 10% are the noradrenaline-secreting type. [2] Adrenaline has many benefits. Physiologically, adrenaline can increase the contraction of the heart muscle, causing dilation of blood vessels in the skeletal muscle and liver as a counterregulatory hormone, and adrenaline causes glycogenolysis and gluconeogenesis in the liver and skeletal muscle. [3; 4] Adrenaline is used as a bronchodilator, prolonging local anesthesia, to overcome type 1 hypersensitivity reactions in medicine. [5] Stress is all environmental changes that change the optimal steady state. [6] The release of adrenaline from the adrenal medulla is stimulated by nerve impulses originating from the adrenergic nuclei in the hypothalamus after being induced by stress (fight or flight response). [7; 8]

Adrenaline also has an antagonistic effect on insulin function and inhibits the entry of blood glucose into peripheral tissues [9]. It aims to mobilize fuel from storage to be oxidized by cells to meet the increased energy needs in acute and chronic stress. This action of adrenaline triggers maximal gluconeogenesis and interferes with glucose uptake into peripheral tissues, resulting in hyperglycemia. [10] For this reason, researchers are interested in examining the relationship of adrenaline administration as a stress response to the blood sugar levels of male Rattus norvegicus rats. The formulation of the problem in this study is: a) How is the blood sugar level of male

Rattus norvegicus rats after being given adrenaline? and b) Is there a relationship between the administration of adrenaline to the increase in blood sugar levels of male Rattus norvegicus rats? The study aimed to a) determine the relationship between adrenaline administration and increased blood sugar levels in male Rattus norvegicus rats.

Theoretical Review

The main adrenaline biosynthetic pathway in the body begins with the amino acid tyrosine. [11] The main catecholamines in the body (adrenaline, noradrenaline, and dopamine) are formed from the hydroxylation and decarboxylation of the amino acid tyrosine. [12] Some tyrosine is formed from phenylalanine, but most are from food. Phenylalanine is an essential amino acid and cannot be synthesized in the body. [13] Tyrosine is converted to Dihydroxy-Phenylalanine (DOPA) by tyrosine hydroxylase and dopamine in the cell cytoplasm. The Vesicular Monoamine Transporter (VMAT) will transport dopamine into the vesicles. [14]

Dopamine will be converted into noradrenaline in the vesicles by Dopamine-Hydroxylase (DBH). [15] The synthesis pathway for noradrenaline is identical to that for noradrenaline-producing neurons, namely noradrenergic neurons. [16] Some neurons in the brain and medulla contain the enzyme Phenylethanolamine-N-Methyltransferase (PNMT). The PNMT enzyme catalyzes the conversion of noradrenaline to adrenaline. Noradrenaline exits the vesicles, enters the cells, is converted into adrenaline, and then enters the vesicles again for storage. [17]

Stress-induced adrenaline release originates from the adrenergic nuclei in the hypothalamus and mediates nerve impulses. Nerve impulses affect acetylcholine which is transported from the cytoplasm into the vesicles by the vesicle-associated transporter. Acetylcholine is released from nerve endings when Ca2+ channels open, allowing Ca2+ influx. 1,10 Ca2+ releases adrenaline from adrenal medulla granules into the extracellular space through exocytosis. [18] The effects of the hormone adrenaline can be seen when the body is in a "fight or flight" condition, namely the body's fight-or-flight physiological response when exposed to events that threaten survival. [19]

Adrenaline and noradrenaline act on the A and B adrenergic receptors (adrenoceptors). Noradrenaline has a greater affinity for adrenoceptors A and adrenaline for adrenoceptors B. Adrenoceptors A1 are present in smooth muscle and the heart, while adrenoceptors A2 are present in the central nervous system, pancreas, and nerve endings. Adrenoceptor B1 is present in the heart and juxtaglomerular of the kidney. B2 adrenoceptors are present in bronchial smooth muscle and skeletal muscle. Adrenoceptors B3 is found in adipose tissue. [20] Adrenaline increases the force and speed of heart muscle contraction, which is mediated by B1 receptors. Adrenaline causes the dilation of blood vessels in skeletal muscle and liver muscle via B2 receptors. [21] Adrenaline affects blood glucose levels as it causes glycogenolysis. It is because adrenaline activates phosphorylase, and through adrenergic receptors A, intracellular Ca2+ levels increase. [22] The effect of adrenaline that causes glycogenolysis is mediated by the Phosphatidylinositol Bisphosphate (PIP2)-Ca2+ signal transduction system. The signal is transmitted from the adrenaline receptor to phospholipase C. Phospholipase C hydrolyzes PIP2 to form diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 will stimulate the release of Ca2+ from the endoplasmic reticulum. Ca2+ and DAG together activate protein kinases. [23]

Ca2+ binds to the calmodulin protein to form a Ca2+-calmodulin complex, then is brought into the cell to unite. Calcium that binds to calmodulin will activate the inactive phosphorylase kinase and make the enzyme active. Active phosphorylase kinase will phosphorylate glycogen phosphorylase b, so glycogen degradation is active. Protein C, calcium-calmodulin protein kinase, and phosphorylase kinase all phosphorylate glycogen synthase and decrease its activity. [24]

Adrenaline will stimulate glucose to be released into the blood vessels. Effects on skeletal muscle, adrenaline stimulates the breakdown of glycogen. In adipose tissue, adrenaline stimulates lipolysis by increasing hormone-sensitive lipase activity. These effects significantly impact blood sugar levels and can cause hyperglycemia if stressful conditions last long. [25] Glucose is a universal fuel for human cells and a carbon source for synthesizing other compounds. Glucose is also a precursor for synthesizing other sugars, which are necessary for forming certain compounds. Glucose can also be converted into fats, amino acids, and nucleic acids. [26] Glucose also plays a role in metabolic homeostasis. It is determined by the fact that many tissues (e.g., the brain, red blood cells, and working skeletal muscles) depend on glycolysis to meet their energy needs. Energy metabolism is carried out quickly and continuously to meet the needs of Adenosine Triphosphate (ATP). Therefore blood glucose levels need to be maintained. If blood glucose levels are not maintained, adverse things will arise, including hyperosmolar effects to diabetes mellitus. The main hormones that play a role in maintaining blood glucose levels are insulin and glucagon. Insulin is the main anabolic hormone in the body. Insulin is secreted to promote the use of glucose for fuel and the storage of glucose as fat and glycogen. The hormone glucagon works as an antagonist of the hormone insulin. Glucagon promotes the formation of glucose through glycogenolysis and gluconeogenesis. [27] In addition to glucagon, adrenaline (a "fight or flight hormone") has an effect opposite to insulin. [28]

After eating a high-carbohydrate meal, blood glucose levels increase from a fasting blood glucose level of about 80-100 mg/dL to about 120-140 mg/dL over 30 minutes to 1 hour. Blood glucose levels will fall again within 2 hours after eating. Glucose levels increase with digestion and absorption of glucose from food. In normal individuals, the increase in blood glucose levels after eating is no more than 140 mg/dL. The liver will oxidize glucose to meet energy needs immediately after eating. Excess glucose is converted into stored fuel and stored in the liver. In the liver, glucose is converted into fatty acids and then glycerol groups which react to form triacylglycerols. This triacylglycerol will be packaged in Very Low-Density Lipoprotein (VLDL). Insulin activates a phosphatase which stimulates glycogen synthase and inhibits the breakdown of glycogen. [29] In muscle, glucose is converted to glycogen through glycogenesis, which insulin stimulates in a resting state. In adipose tissue, insulin stimulates the transport of glucose into cells. This glucose provides energy for cells and forms glycerol groups for triacylglycerol synthesis. Glucose can be converted into fatty acids in adipose tissue. [30]

During fasting, the state of blood glucose decreases so that insulin decreases and glucagon increases. The increased hormone glucagon stimulates the liver to break down stored glycogen through glycogenolysis and form glucose through gluconeogenesis. It happens to maintain blood glucose levels. Glucagon regulates glycogen metabolism through intermediaries of cyclic adenosine monophosphate (cAMP) and protein kinase A. Glucagon activates adenylate cyclase, which causes

cAMP levels to increase through protein G. cAMP binds to the regulatory subunit of protein kinase A, causing protein kinase A to become active. Active protein kinase A will phosphorylate the phosphorylase kinase enzyme to become active. Phosphorylase catalyzes the phosphorylation of glycogen, yielding glucose 1-phosphate and converting it to glucose 6-phosphate. Dephosphorylation of glucose 6-phosphate by glucose 6-phosphatase produces free glucose, which will be circulated into the blood. [31]

Gluconeogenesis takes place during fasting. The non-carbohydrate precursors in gluconeogenesis are lactic acid, amino acids, or glycerol. Pyruvate can form oxaloacetate with the help of pyruvate carboxylase. Phosphoenolpyruvate is formed from the release of CO2 added to pyruvate. Oxaloacetate undergoes decarboxylation to form malate and can penetrate the mitochondrial membrane and enter the cytosol. In the cytosol, phosphoenolpyruvate forms glyceraldehyde 3-phosphate. For every two molecules of glyceraldehyde 3-phosphate, one is converted to DHAP. Through the aldolase reaction, fructose 1,6-bisphosphate is formed. The enzyme fructose 1,6-bisphosphatase liberates inorganic phosphate from fructose 1,6-bisphosphatase to form fructose 6-phosphate. Fructose 6-phosphate is converted to glucose 6-phosphate by isomerase, which is used for glycolysis. Glucose 6-phosphatase breaks the P1 bond and releases glucose into the blood. [32]

Research Method

This study used healthy male Rattus norvegicus rats of the Spraque Dawley strain, aged 24 weeks, weighing 250-300 gr. Mice were obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. Before and during treatment, the health of the rats was continuously monitored so they would not get sick. Mice were given food and drink ad libitum. The cage is kept clean, and the light is set to 12 hours of light and 12 hours of darkness. In addition, other matters are also considered following the code of ethics of the commission for handling and using experimental animals. The tools used in this study were a glucose meter, one cc syringe, body weight scales, and 70% alcohol cotton. The materials used in this study were adrenaline ampoules and 0.9% NaCl. Experimental animals are used in research to the maximum extent possible by implementing the principles of animal welfare. Before the research was carried out, it was submitted, and a certificate of passing the ethical study on the use of experimental animals was obtained from the research ethics assessment team at the Faculty of Medicine, Indonesian Christian University. The research was conducted as an in vivo experimental study using male Rattus norvegicus rats, divided into four groups with six rats in each group. Group 1 (K1) is the control group for rats that did not fast for 12 hours and were not given adrenaline injections. Group 2 (K2) was the control group for rats that fasted for 12 hours and were not given adrenaline injections. Group 3 (K3) is the treatment group for rats that were treated with an adrenaline injection and did not fast for 12 hours. Group 4 (K4) is the treatment group for rats that were treated with an adrenaline injection and fasted for 12 hours. The research was conducted at the Animal Management Unit Laboratory of the Bogor Agricultural Institute, West Java. The study took place from May 2019 to November 2019. Adrenaline was diluted with 0.9% NaCl and injected intraperitoneally using a one cc syringe at a dose of 0.6 mg/kgBB3. Administration of adrenaline can increase blood glucose levels in rats which are reversible. Adrenaline will stimulate glycogenolysis and gluconeogenesis. Conditions of hyperglycemia is a

condition that is expected from the purpose of this study. The parameters examined in the study were measurements of blood sugar levels in male Rattus norvegicus rats by pre-test and post-test. After all the data was collected, the research data analysis was carried out. Data analysis used the Statistical Package and Service Solutions (SPSS) program with a significance level of $p<0.05$.

Result and Discussion

Based on the research conducted, the following results were obtained:

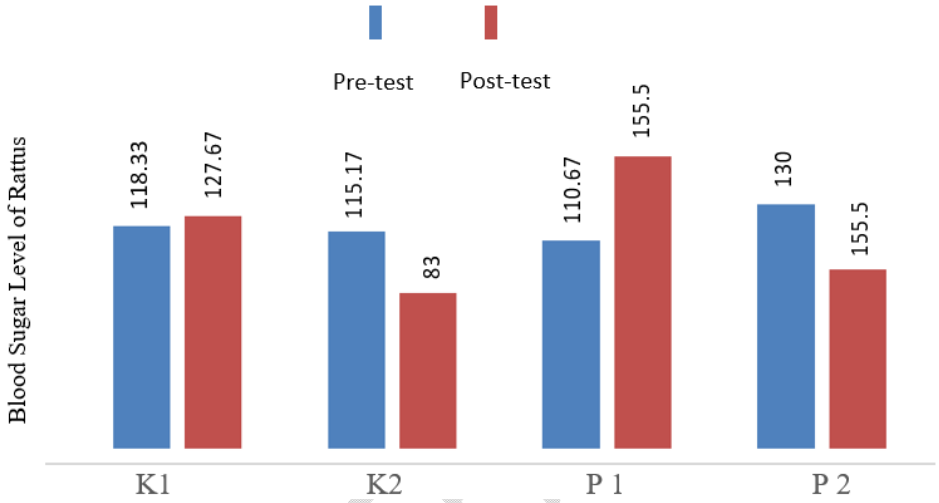

Diagram 1. Blood Sugar Levels of Rattus norvegicus Male Rats Pre-test and Post-test

In diagram 1, the pre-test blood sugar level in control group 1 was 118.33 mg/dL, and the post-test blood sugar level was 123.67 mg/dL. The results of the pre-test blood sugar in the control group 2 were 115.17 mg/dL, and the post-test blood sugar levels were 83 mg/dL. The results of the pre-test blood sugar levels in treatment group 1 were 110.67 mg/dL, and the post-test results were 155.5 mg/dL. The results of the pre-test blood sugar levels in treatment group 2 were 130 mg/dL, and the post-test results were 95.17 mg/dL. In control group 1, which did not fast and was not injected with adrenaline 0.6 mg/kg, there was no significant difference ($p>0.05$) between pre-test and post-test blood sugar levels. These results were probably due to the absence of stress stimuli in the rats, which caused the pre-test and post-test blood sugar not to differ much (118.33 mg/dL and 123.67 mg/dL).

Control group 2, rats that were fasted and not injected with adrenaline, had a significant difference ($p<0.05$) in pre-test and post-test blood sugar levels. These results follow research conducted by Alfin (2019), which states that blood glucose levels will decrease during fasting so that insulin production will decrease. When the body is fasting, the liver releases glucose into the blood so that the tissues that need glucose do not experience a lack of energy. [33] Adrenaline administration of 0.6 mg/kg in treatment group 1, rats that did not fast, gave a significant difference ($p <0.05$) in the pre-test and post-test blood sugar levels. The body is not in a fasted state; after food is digested and absorbed, blood glucose levels rise until it reaches a peak, then slowly decrease towards normal. It happens because of the hormone insulin, which helps glucose to enter the peripheral tissues and not circulate freely in the blood.

When the body is exposed to a stressor, a response will appear, controlled by the central nervous system. This response will affect the body through three systems: the autonomic nervous system, the neuroendocrine system, and the immune system. The autonomic nervous system regulates metabolism. The autonomic system has two working nerve pathways, parasympathetic innervation, and sympathetic innervation, which prepare the body for stress conditions, such as increasing blood flow to the skeletal muscles. One catecholamine hormone is adrenaline, inhibiting insulin action and stimulates gluconeogenesis and glycogenolysis. [13] Adrenaline administration of 0.6 mg/kg BW in treatment group 2, the fasted rats, gave a significant difference ($p<0.05$). However, the pre-test blood glucose levels were higher than the post-test (130 mg/dL and 95.17 mg/dL). The results follow research conducted by Faulenblach (2011), which showed that stress does not increase blood glucose levels during fasting. This result is because blood glucose during fasting is low, and the stress experienced cannot make blood glucose levels return to homeostasis. [34]

Research conducted by Ugahari (2016) found that excessive stress did not raise blood glucose levels during fasting. It occurs in long fasting (12 hours or more), which will reduce the intake of carbohydrates or glucose so that the body is in a state of hypoglycemia. Glycogenolysis in the liver is an important process to meet glucose needs in a fasting state for 12 hours or more. [36]

Conclusion

Administration of adrenaline increased blood sugar levels in the group of rats that did not fast, but administration of adrenaline did not increase blood sugar levels in the group of rats that fasted. It is suggested that further researchers conduct further research to analyze rats' cortisol levels, lipid profiles, and stress index.

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