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Levels of glycolytic intermediate metabolites in brain cells of rats under conditions of energy drink consumption

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Abstract. Energy drinks are popular among young people to increase performance and attention. However, their impact on metabolic processes and physiological functions has led to restrictions on their consumption in many countries. The study aimed to determine the levels of glycolytic intermediate metabolites in rat brain neurons under conditions of energy drink consumption. The study was conducted on sexually mature Wistar rats weighing 180-200 g. The animals were divided into groups, and biological material was collected for experimental purposes. Brain homogenate was prepared using a homogeniser and a cold extraction medium in a ratio of 1/9. In the obtained brain homogenate and blood serum, the concentrations of glucose, pyruvate, lactate, and the activity of the glycolytic enzyme – lactate dehydrogenase were determined. It was investigated that the consumption of an energy drink by animals led to an increase in blood glucose levels and the development of persistent hyperglycaemia. Regarding the glucose content in the brain, there was an observed increase in its utilisation by neurons. It was also established that in brain cells, the concentration of lactate (the final product of anaerobic glycolysis) and the activity of the key glycolytic enzyme, lactate dehydrogenase, increased. A decrease in the level of the glycolytic intermediate metabolites, pyruvate, was investigated in neurons. However, in blood serum, opposite changes in pyruvate levels were observed: at the beginning of the experiment, the level of pyruvate increased relative to the intact control with a tendency to normalise in the later experimental periods. By changing the level of glycolytic intermediate metabolites in the brain homogenate, it is possible to determine the course of metabolic processes and the intensity of the energy supply of brain cells

Keywords: lactate; pyruvate; brain; lactate dehydrogenase; carnitine; caffeine

INTRODUCTION

The study of the effects of energy drinks is becoming increasingly relevant today. This is because the consumption of these drinks elicits conflicting opinions: interest from young people and warnings from doctors. Energy drinks are classified as non-alcoholic, highly carbonated beverages containing carbohydrates (glucose, sucrose), alkaloids of the xanthine group (caffeine, theobromine, theophylline), extracts of guarana and ginseng, carnitine, vitamins (A, B_1, B_2, B_6, B_{12}, C, P), amino acids (taurine), and macro- and microelements: magnesium, potassium, manganese, sodium, iron, chlorine – all of these are considered stimulants for the body. Therefore, they are used to prolong the period of wakefulness, relieve fatigue, increase productivity, and enhance memorisation during exams by students, among other purposes. Most teenagers do not adhere to consumption recommendations. According to modern literature sources, the most powerful psychostimulant in the drink is caffeine, which reduces feelings of fatigue, improves cognitive function, increases heart rate, and has a diuretic effect. Under the influence of caffeine, not only mental activity

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but also physical activity increases, and the reaction-response to external influences is enhanced. The period of stimulation is followed by fatigue.

I. Klishch *et al.* [1] point out that under the influence of caffeine, such metabolic processes as glycolysis, lipolysis, general metabolism and oxygen demand accelerate, and hyperglycaemia may occur. J.P. Higgins *et al.* [2] identify another component of the drink – theobromine, but it has a less stimulating effect. It is an alkaloid of the purine series, and in its physical properties, it is transparent crystals that are bitter in taste. The therapeutic dose of theobromine is significantly higher than that of caffeine. This compound has a relaxing effect on smooth myocytes. Carnitine (a component of the drink) also enhances carbohydrate metabolism, has a positive effect on cardiovascular activity, and is sometimes used in medical practice. In her study, I. Yastremska [3] notes that L-carnitine acts as an active regulator of intermediate metabolism and energy supply processes. Therefore, the use of L-carnitine in the treatment of myocardial infarction, in addition to its pronounced energy-producing activity and antioxidant effect, had an effective impact on the endothelial function of blood vessels. Guarana and ginseng are natural stimulants with different effects, they also reduce fatigue and increase mental and physical activity. B vitamins are important cofactors in biochemical transformations but do not possess “energy-giving” properties. There are serious safety concerns about the safety of these products, especially with such a composition. In scientific publications, I.M. Nadeem *et al.* [4] mention disorders of the nervous system as a result of the consumption of energy drinks (irritability, fatigue, sleep disturbances, cramps). Therefore, studies of the effect of energy drinks on metabolic processes in the brain cells that ensure the overall functioning of the nervous system and occur with the loss of energy resources will be important. The intensity of energy metabolism is a leading factor limiting brain activity. The brain obtains energy (ATP) as a result of the aerobic breakdown of glucose (glycolysis), which ensures normal brain function: the conduction of excitation by nerve fibres, the synaptic transmission of signals, the specific activity of nerve centres, and the molecular mechanisms of integrative brain functions – memory, thinking, consciousness. Kh. Partsei [5] highlights information about the effect of energy drinks on carbohydrate metabolism in erythrocytes, in particular, an increase in glucose catabolism, as evidenced by an increase in lactate concentration and LDH activity. The authors Kh. Partsei *et al.* [6] investigated the changes in the pro- and antioxidant systems of rat erythrocytes under conditions of energy drink consumption. Their study presents the effect of energy drinks on free radical oxidation processes, which are accompanied by the activation of lipid peroxidation and protein peroxidation, which leads to a violation of the structural and functional ability of erythrocyte cell membranes, as well as an increase in the activity of superoxide dismutase. There is no data on carbohydrate metabolism in the brain. The purpose of the study was to investigate changes in carbohydrate metabolism in brain cells of experimental animals as a result of energy drink consumption.

MATERIALS AND METHODS

The experimental work was carried out at the “Centre of Bioelementology” of Ivano-Frankivsk National Medical University (accreditation certificate – CDL No. 037/19 of 13 June 2019) from October to December 2021. The study involved determining the effects of energy drinks on the metabolic processes of sexually mature male rats weighing 180-200 grams. A total of 56 animals were used in the experiment. The experimental animals were kept on a standard vivarium diet under appropriate temperature and lighting conditions (12-hour light cycle), adhering to all requirements and recommendations [7, 8]. Before the start of the experiment, all animals underwent a 10-day acclimatisation period to avoid the influence of stress factors on the measured parameters. At each stage of the experiment, the weight of the animals was recorded. The volume of the energy drink was calculated individually for each laboratory rat, based on the threshold limit value for a healthy adult human (indicated on the drink packaging) recalculated per kg of animal weight. The energy drink was administered orally. Experimental animals were divided into groups, the experimental model is presented in Table 1. All animals had free access to drinking water. The composition of the studied energy drink includes the following substances: purified water, sugar, carbon dioxide, citric acid, sodium citrate, taurine, preservatives, potassium sorbate, sodium benzoate, sugar colour, flavourings, caffeine, inositol, vitamins (niacin, B_6, B_{12}), guarana extract, and the antioxidant ascorbic acid.

Table 1. Distribution of animals in groups

Animal groups	Number of animals	Influence	Term of biomaterial collection
Group 1 (intact control)	28	rats that consumed water	1st, 10th, 20th, 30th, after discontinuation of the drink
2nd group (experimental)	7	rats that consumed the energy drink for 30 days	1 day, after discontinuation of the drink
3rd group (experimental)	7	rats that consumed the energy drink for 30 days	10 days, discontinuation of the drink
4th group (experimental)	7	rats that consumed the energy drink for 30 days	20 days, discontinuation of the drink
5th group (experimental)	7	rats that consumed the energy drink for 30 days	30 days, discontinuation of the drink

Source: compiled by the authors

Before collecting biological samples, the experimental animals were anesthetised with sodium thiopental. Since the sampling was conducted under identical conditions for all groups, the effects of the collection methods on glycolytic intermediate metabolites and the anaesthetic effect are neutralised. The brain and blood of experimental animals were collected. Brain homogenate was prepared using a homogenizer and a cold extraction medium in a 1:9 ratio. In the obtained brain homogenate and blood serum, the levels of glucose (glucose oxidase method), pyruvate (by the amount of 2,4-dinitrophenylhydrazone derivatives), lactate (by reaction with para-oxydiphenylene), and lactate dehydrogenase (LDH) activity were determined. The enzymatic activity of LDH was determined spectrophotometrically. Statistical analysis of the results was performed using the STATISTICA 8 program, taking into account the Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Glucose is the primary energy source for brain cells, so even slight fluctuations in glucose levels can disrupt metabolic transformations in brain neurons [9]. The brain uses 70% of the glucose synthesised in the liver and that obtained from food [10]. In neurons, approximately 90% of glucose is oxidised to CO_2 and H_2O in the Krebs cycle (aerobic glycolysis), 5% is spent in the process of anaerobic glycolysis with the formation of lactate, and 5% in the reactions of glycogen, sphingolipids, cerebrosides, sulfatides, glycoproteins, and neurotransmitter synthesis [11]. As a result of the conducted research, changes in glucose content were observed as early as the 1st day after 30 days of consuming the energy drink – the glucose content increased by 19.38% compared to the intact control; on the 10th day, the glucose concentration remained slightly elevated by 6.3%; on the 20th day, the glucose level was within the normal range. Such changes in glucose indicators (Fig. 1) are predictable, since according to the literature, the effect of all components of energy drinks is aimed at achieving an energy boost effect [12]. However, already on the 30th day of discontinuing the energy drink, the glucose content decreased by 11.59% compared to the control. This may lead to the exhaustion of neurons, feelings of fatigue, apathy, and other disorders of the nervous and other systems. The author Kh. Partsei [5] presented a study of an increase in glucose content in erythrocyte hemolysate under the influence of an energy drink by 1.8 times, and after discontinuation of the drink, the glucose level decreased by 1.2 times.

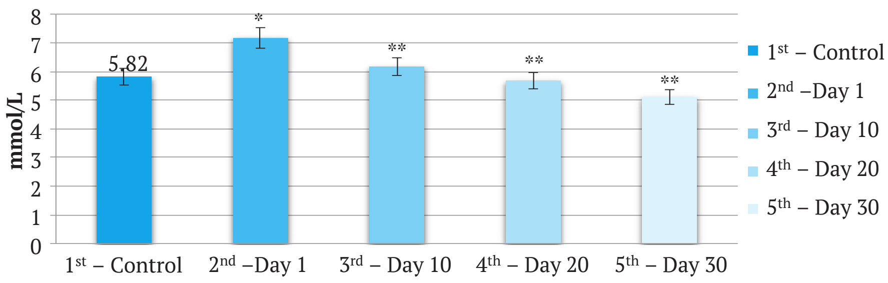

Figure 1. Changes in glucose levels in the brain homogenate in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

The level of glucose in the blood is also influenced by carnitine, a component of the drink. In the studies of H. Fathizadeh *et al.* [13], it is noted that the treatment of patients with carnitine led to a decrease in the level of glucose and insulin in fasting plasma. At the same time, this study examined the level of glucose in blood serum (Fig. 2), which made it possible to establish the development of persistent hyperglycaemia throughout the entire experimental period. In rats of the 2nd group, the glucose content in blood serum increased by 21.78% compared to intact animals and remained elevated throughout the entire study period: in the 3rd group – by 17.94%, in the 4th – by 11.27%, in the 5th – by 6.79%.

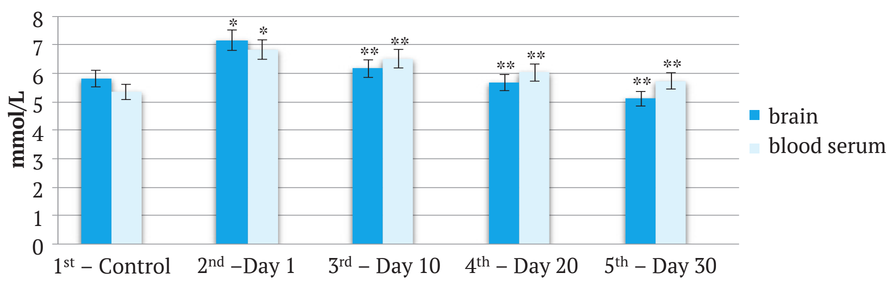

Figure 2. Comparative analysis of changes in glucose levels in brain homogenate and blood serum in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

Comparative analysis of glucose content (Fig. 2) in brain homogenate and blood serum showed an increase in the second experimental group of animals. Glucose levels in animals of the third group decreased in both studied substrates compared to the second group but remained elevated relative to the intact control (1st group). Initially, glucose levels in the brain decreased. By the 20th day of the experiment, the glucose level in the brain normalised, but in the blood serum, it remained elevated by 1.12 times. In the 5th experimental group, a decrease in the glucose level in the brain homogenate was observed, but in the serum, it exceeded the norm by 1.07 times. It is worth noting that the glucose level, after consumption of the energy drink, immediately increased in the brain and blood serum, but after discontinuation of this drink, the glucose level in the brain homogenate was lower than the intact control, while in the blood serum, it remained elevated.

To understand glucose metabolism, it is important to study the products of intermediate metabolism, in particular pyruvate and lactate. Pyruvic acid (pyruvate) is formed during glycolysis and during the conversion of lactate under the action of lactate dehydrogenase [14]. Under aerobic conditions, pyruvate is oxidised to acetyl-CoA, can enter the Krebs cycle and provide energy for the body or be used for other metabolic pathways (in particular, for the synthesis of fatty acids or cholesterol) [15]. The concentration of pyruvate in the brain (Fig. 3) on the 1st day, after discontinuation of the energy drink, was 0.231 ± 0.0012 (mmol/L), which is 10.8% lower than the intact control indicator.

On the 10th and 20th days, the level of pyruvic acid remained decreased by 12.33%, and on the 30th day – by 4.12% with a tendency to normalise (0.248 ± 0.02 mmol/L). However, in the studies of Kh. Partsei [5], the content of pyruvate in erythrocyte hemolysate steadily increased by 3.9 times compared to the intact control during the consumption of the energy drink and decreased in the subsequent periods of the study (10th, 20th, and 30th days of the experiment). R. Ostapiv [16], in his studies of the effect of taurine on energy processes in cells, notes that when a smaller dose of taurine (40 mg/kg) is administered, the glucose content decreases relative to the control group of animals, and when a larger dose is administered (100 mg/kg), the glucose level increases. Taurine is a derivative of sulphur-containing amino acids, present in energy drinks, and can increase tissue sensitivity to glucose.

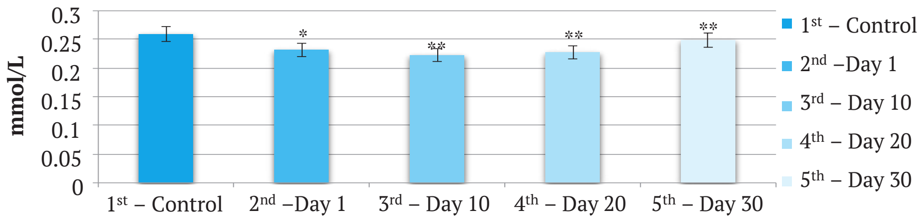

Figure 3. Changes in pyruvate levels in brain neurons in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 (control) of animals ($p < 0.01$); ** – significant compared to the data of group 2 of animals ($p < 0.01$)
Source: compiled by the authors

O. Remennyk [17] believes that an increase or decrease only in pyruvate in the blood is not a diagnostic sign; usually, the ratio of lactate to pyruvate is determined, which characterises the state of glycolytic and oxidative conversion of carbohydrates. It is well known that in cells with limited oxidative capacity or under hypoxic conditions, the tricarboxylic acid cycle is blocked, and pyruvate, under the action of lactate dehydrogenase, is converted to lactic acid, lactate. In this case, only 2 ATP molecules are formed [18] and hyperlactatemia occurs. The reasons for its appearance may be congenital diseases (type I glycogenosis), vitamin B_1 deficiency, administration of adrenaline, sodium nitroprusside into the blood, in case of alkalosis, epilepsy, the intake of too much fructose, sorbitol or xylitol [19]. An increase in the level of pyruvic acid in tissues may indicate a disruption of the balance between oxygen supply systems to tissues and the need for it. It has been established that the level of pyruvate in the blood increases as a result of decompensation for hypoxic conditions [20]. In blood serum (Fig. 4), the content of pyruvate increased on the 1st day after discontinuation of the energy drink by 29.6%, on the 10th day – by 24%, on the 20th day – remained elevated by 13.6% and with a tendency to normalise the level of this indicator on the 30th day.

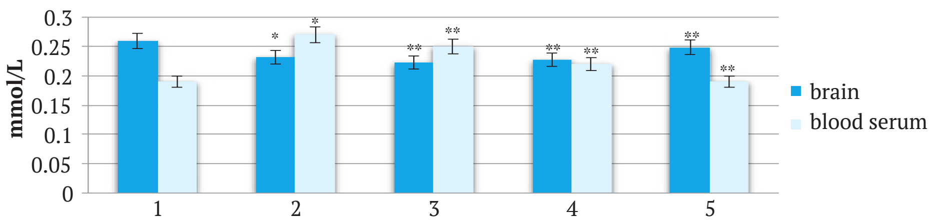

Figure 4. Comparative characteristics of changes in pyruvate levels in brain homogenate and blood serum in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

Regarding the comparative characteristics of pyruvate content in brain homogenate and blood serum, the changes are observed to be in different directions (Fig. 4). In the brain homogenate, in almost all experimental groups of animals, the level of pyruvate is lower than the intact control, only in the last period of the experiment is there a tendency towards normalisation. Regarding the pyruvate content in blood serum, an increase in this indicator was noted in the 2nd, 3rd, and 4th groups of animals and a return to normal in the 5th group relative to the intact control.

The study of lactate (Fig. 5) in brain homogenate showed an increase in this indicator in the 2nd group of animals by 20.39%, in the 3rd and 4th groups – by 13.37% and 4.98% respectively, and in the 5th group, the lactate content stabilised relative to the control group of animals. In their article, Kh. Partsei [5] also presents an increase in lactate content in erythrocyte hemolysate in the 2nd, 3rd, 4th, and 5th experimental groups by 1.9 times, 1.6 times, 1.2 times, and 1.1 times, respectively, under the influence of the energy drink.

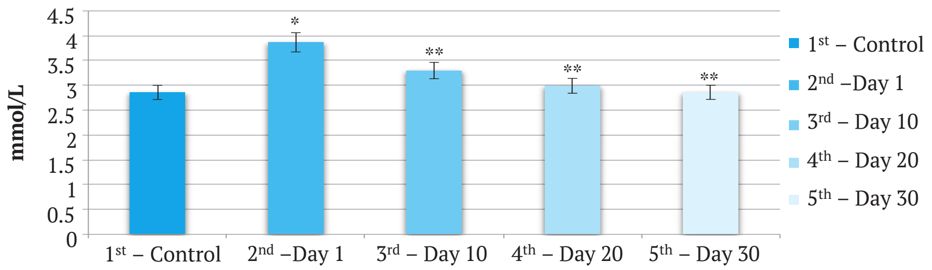

Figure 5. Changes in the level of lactate in brain neurons in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

A similar trend of increasing lactate and pyruvic acid content was observed by G. Khitry *et al.* [21] in their study of changes in carbohydrate metabolism metabolites in rats under general hypothermia. High lactate content can be observed during hypoxia, diabetes mellitus, excessive intake of sucrose and other substances, as well as with impaired lactate elimination by the liver (60%) and kidneys (30%). An increase in lactate concentration above 2 mmol/L is a diagnostic indicator of sepsis, and an increase above 4 mmol/L serves as a criterion for multiple organ dysfunction syndrome [22]. Lactate is a product of anaerobic glycolysis and is formed from pyruvate under the action of the enzyme lactate dehydrogenase with limited oxygen supply [23]. Reduces the activity of the enzyme inosine, a component of the energy drink [24]. R. Ostapiv & V. Manko [25] studied the effect of taurine on carbohydrate metabolism; the results of their research indicate that taurine regulates calcium metabolism and increases the activity of lactate dehydrogenase, therefore, it can be effective during intense muscular work.

The conducted studies of lactate dehydrogenase in brain homogenate showed a significant ($p < 0.05$) increase of 49.78% in the brains of rats in the 2nd group. As seen in Figure 6, the enzyme activity remained high (by 43% compared to the intact control) for 10 days after discontinuation of the drink, and on the 20th and 30th days, there was a tendency towards normalisation. In the study of Kh. Partsei [5], research on the activity of lactate dehydrogenase in erythrocyte hemolysate under the influence of an energy drink is presented. According to the author, the activity of lactate dehydrogenase increased by 2.6 times relative to the intact control, on the 10th, 20th, and 30th days, the enzyme activity remained elevated by 1.4 times, 1.9 times, and 2.1 times, respectively, compared to the indicators of the second group. According to the results of studies on the effect of taurine on energy metabolism, R. Ostapiv & V. Manko [25] reported an increase in lactate dehydrogenase activity in rat erythrocytes – by 59%, and in the brain – by 80% compared to the control. The authors suggest that such changes are possible with a limited oxygen supply. Prolonged administration of taurine leads to a decrease in the mass of organs, compared to the control, but the mass of the brain increases, which is explained by the positive effect of taurine on energy supply.

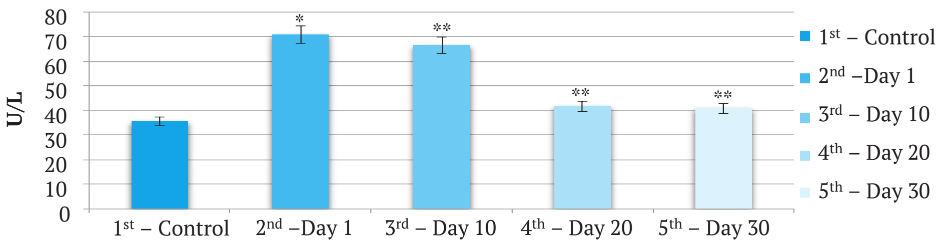

Figure 6. Changes in the activity of lactate dehydrogenase in brain cells in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

In their research, E. Choo *et al.* [26] note that the consumption of caffeine leads to an increase in blood glucose levels. According to the results of this study, the level of glucose in blood serum and brain homogenate also increases under the conditions of consuming an energy drink. According to the article by S.M. Seifert *et al.* [27], an overdose of caffeine or energy drinks leads to loss of sleep, increased blood pressure, nausea, nervousness, muscle tone loss, cramps, changes in heart rhythm, and feelings of anxiety. Thus, the consumption of an energy drink by experimental animals leads to changes in some intermediates and enzymes of glycolysis (increased glucose and lactate content, increased lactate dehydrogenase activity, but decreased pyruvate level) in brain neurons and blood serum, which are described in the following conclusions.

CONCLUSIONS

This study aimed to investigate the impact of energy drinks on the organism of experimental animals, specifically focusing on carbohydrate metabolism parameters over 30 days of energy drink consumption and a 30-day post-experiment period. The main objective of this study was to identify changes in the content of glucose and intermediate glycolytic metabolites (pyruvate, lactate) in brain cells and serum. To assess the energy supply of neurons and the course of oxidative processes, changes in lactate dehydrogenase activity were studied. During the experiment, an increase in glucose concentration in the brain cells of experimental animals was found from 5.82 ± 0.02 mmol/L (intact control) to 7.17 ± 0.03 mmol/L on the 1st day, after 30 days of energy drink consumption. The following periods of the study were accompanied by the normalisation of this indicator to 5.11 ± 0.008 mmol/L (30th day, after discontinuation of this drink consumption). An increase in glucose content can lead to glycosylation of various structures in the brain. The research on carbohydrate metabolism metabolites in brain homogenate indicates a consistent decrease in pyruvate concentration throughout the entire experimental period, approximately by 10%. At the same time, the level of lactate, under the conditions of consuming an energy drink in rat brain cells, steadily increased from 2.85 ± 0.036 mmol/L to 3.86 ± 0.041 mmol/L. The opposite nature of changes in glycolysis intermediates – rising lactate alongside decreasing pyruvate – indicates an intensification of anaerobic glucose conversion. Confirmation of this is the increase in lactate dehydrogenase activity by 49%. Such changes are not typical for the brain and may indicate impaired functioning of the nervous system. Therefore, the perspective of further research will be to determine the activity of key enzymes of aerobic glycolysis, namely the enzymes of the tricarboxylic acid cycle (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, mitochondrial malate dehydrogenase, and Na^+/K^+ – ATPase). These enzymes will reflect the course of oxidative processes in the brain cells of experimental animals, which will help predict the effects of energy drinks on the human body to some extent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Рівень проміжних інтермедіатів гліколізу у клітинах головного мозку щурів за умов споживання енергонапою

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Анотація. Енергетичні напої популярні серед молоді як засоби для підвищення працездатності та уваги. Однак їх вплив на метаболічні процеси та фізіологічні функції призводить до обмежень їх вживання в багатьох країнах. Метою дослідження було визначення рівня проміжних інтермедіатів гліколізу у нейронах мозку щурів за умов споживання енергетичного напою. Дослідження було проведено на статевозрілих щурах лінії Вістар, масою 180-200 г. Тварин було розпределено по групах. З експериментальною метою здійснювали забір біоматеріалу. Гомогенат головного мозку готували з використанням гомогенізатора та охолодженого середовища виділення в співвідношенні 1/9. В отриманому гомогенаті мозку та сироватці крові проводили визначення концентрації: глюкози, пірувату, лактату та активності гліколітичного ензиму – лактатдегідрогенази. Було досліджено, що споживання енергетичного напою тваринами призводило до підвищення рівня глюкози в крові і виникнення стійкої гіперглікемії. Щодо вмісту глюкози в головному мозку спостерігалось посилене її використання нейронами. Також було встановлено, що у клітинах головного мозку зростала концентрація лактату (кінцевого продукту анаеробного гліколізу) та активність ключового ензиму гліколізу – лактатдегідрогенази. Було досліджено у нейронах зниження рівня проміжного інтермедіату гліколізу – пірувату. Проте, в сироватці крові досліджено протилежні зміни рівня пірувату: на початку експерименту рівень пірувату підвищувався відносно інтактного контролю з тенденцією до нормалізації в крайні дослідні періоди. За зміною рівня проміжних метаболітів гліколізу в гомогенаті мозку можна визначати перебіг метаболічних процесів та інтенсивність енергозабезпечення клітин головного мозку

Ключові слова: лактат; піруват; головний мозок; лактатдегідрогеназа; карнітин; кофеїн