БІОХІМІЯ, БІОТЕХНОЛОГІЯ, МОЛЕКУЛЯРНА ГЕНЕТИКА

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PECULIARITIES OF TYROSINE METABOLISM IN THE RAT LIVER UNDER THE CONDITION OF PROTEIN DEFICIENCY

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In the present study, a tyrosine content in liver and enzymatic activities of its metabolism: tyrosine aminotransferase, 4-hydroxyphenylpyruvate dioxygenase, aldehyde dehydrogenase (ALDH3A1) were investigated under the conditions of alimentary protein deprivation. The experiments were performed on white rats with a body weight of 100-150 g aging 2.5-3 months. The animals were divided into two experimental groups: I – animals receiving a full-value semi-synthetic ration (C); II – animals receiving a low-protein ration (LPR). In order to simulate the low-protein diet, the animals received a diet containing 4.7% protein, 10% fat and 85.3% carbohydrates for 28 days, calculated according to the American Institute of Nutrition's recommendations. Determination of tyrosine in deproteinized by 6% sulfosalicylic acid extracts of the liver tissue was performed using the automatic analyzer of amino acids T-339 (Microtechnology, Czech Republic). The enzyme activity was determined by spectrophotometric method – tyrosine aminotransferase by the amount of 4-hydroxybenzaldehyde, which has a maximum absorption at 331 nm, 4-hydroxyphenylpyruvate dioxygenase – by the intensity of colored product formation at λ 336 nm, ALDH3A1 activity was measured at 340 nm wavelength. It was established a 5-fold depletion of the tyrosine pool and a 2-fold reduction of the tyrosine aminotransferase activity, which catalyzes the formation of the first transformation of the tyrosine – 4-hydroxyphenylpyruvate transformation in the liver tissue under the condition of protein deficiency. Determination of the key enzymes activity of two possible ways of further 4-hydroxyphenylpyruvate transformation has shown that the activity of 4-hydroxyphenylpyruvate dioxygenase, as an enzyme of the homogentisin pathway of tyrosine metabolism, remains at the control level, while the aldehyde dehydrogenase, as the key enzyme of the synthesis pathway of the benzoyl ring of ubiquinone molecule, is by half reduced. The obtained results allow us to conclude that under the conditions of an alimentary protein deficiency tyrosine is predominantly metabolized in the liver by the homogenetistic pathway, which can be considered as a compensatory reaction directed at the maintaining energy metabolism while simultaneously affecting the use of tyrosine as a precursor in ubiquinone synthesis.

Key words: alimentary protein deficiency, liver, mitochondria, tyrosine, ubiquinone, tyrosine aminotransferase, 4-hydroxyphenylpyruvate dioxygenase, aldehyde dehydrogenase

Introduction. Understanding of the protein metabolism pattern under the pathological conditions remains a problem that has not only scientific-theoretical but also practical interest.

At present it is known the protein deficiency occurs not only as a result of an alimentary lack of protein (in case of complete or partial starvation, unvaried ration, prevalence of vegetable proteins in diet), but also due to a number of diseases, even in use of a sufficient amount of full-value protein. Prolonged alimentary protein deprivation is accompanied by the disturbances of the cell cycle, cell differentiation and apoptosis, increased activity of the enzymes involved in the regulation of oxidative status and homeostasis. At the same time, there is a significant reduction in free amino acids content in liver, indicating their important role in organism adaption to the alimentary protein deprivation (Kalhan et al., 2011).

The optimal balance between the content of amino acids in ration and the intensity of their metabolism is a critical factor in maintaining the homeostasis of the whole body (Wu, 2009). Amino acids, especially those with specific metabolic pathways, draw specific attention, in particular aromatic amino acids – phenylalanine and tyrosine.

Tyrosine in the body is originated from the hydrolysis of food protein and synthesis from essential amino acid phenylalanine. About 30% of the resulting free tyrosine is used for synthesis of catecholamines, melanin, and thyroid hormones, while a part of it is used for the renewal of tissue proteins. More than 60% of tyrosine enters the liver where it is oxidized. In the liver, tyrosine is included in the transamination reaction involving the

pyridoxal-dependent enzyme tyrosine aminotransferase to form 4-hydroxyphenylpyruvate. This compound may be metabolized in two ways. The first is homogentisic with the formation of fumarate and acetoacetate, which are used as substrates in energy metabolism reactions. The other way is the tyrosine metabolism to 4-hydroxybenzoate – the precursor of the benzoyl ring of ubiquinone molecule (Syrova et. al., 2014; Rass et al., 2016; Sandip et al, 2016).

Therefore, the aim of the present study was to determine tyrosine concentration and the activities of the enzymes of its metabolism, namely tyrosine aminotransferase (EC 2.6.1.5), 4-hydroxy-phenylpyruvate dioxygenase (EC 1.13.11.27), aldehyde dehydrogenase ALDH3A1 (EC 1.2.1.3) in the rat liver under the condition of dietary protein deprivation.

Materials and Methods. The experiments were performed on white rats with a body weight of 100-150 g and 2.5-3 months of age. Animal maintenance and all manipulations were conducted in accordance with the article 26 of the Law of Ukraine № 3447-IV 21.02.2006 "On the protection of animals from cruelty", "The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986),"General Ethical Considerations for Animal Experimentation" established by First Ukrainian Congress on Bioethics (Kyiv, 2001).

The animals were placed into plastic cages with sand bedding and *ad libitum* access to water. The daily rations were regulated according to principles of pair feeding. The animals were divided into the two experimental groups: I – animals receiving complete semi-synthetic ration for 28 days (C); II – animals receiving low-protein ration, for 28 days (LPR).

The animals of the group I were fed with a standard fodder containing 14% protein (casein), 10% fat, and 76% carbohydrates, balanced by all the essential nutrients. The animals of the group II were fed with isoenergetic fodder containing 4.7% protein, 10% fat, and 85.3% carbohydrates, calculated according to the recommendations of the American Institute of Nutrition (Voloshchuk, Kopylchuk, 2016).

Cervical dislocation was performed under the light ether anesthesia on day 29 of the experiment.

The liver mitochondrial fraction was isolated from the homogenate by differential centrifugation at 0-4°C (Acopova, Sagach, 2004).

The tyrosine content was determined using the automatic analyzer of amino acids T-339 (Microtechnology, Czech Republic), the studied samples were deproteinized by 6% sulphosalicylic acid. The tyrosine content was expressed in mg/g of tissue.

The tyrosine aminotransferase activity was determined by spectrophotometric assay using *n*-hydroxybenzaldehyde, which is formed from the product of the tyrosine deamination to 4-hydroxyphenylpyruvate in an alkaline medium under the influence of air oxygen, and has a maximum absorption at 331 nm (Rain-Guion, Chambon, 1982). The enzyme activity was expressed in nmol/min×mg of protein.

The 4-hydroxyphenylpyruvate dioxygenase activity was determined by spectrophotometric assay at 336 nm as a rate of formation of a complex between boric acid and enol tautomer (Knox, Pitt, 1957).

ALDH3A1 activity was measured in 100 mM Na_2HPO_4 buffer at pH 7.5, with 1.5 mM $NADP^+$ and 1 mM benzaldehyde at 340 nm wavelength (Parajuli et al., 2014). The enzyme activity was expressed in nmol $NADP^+$/min×mg of protein.

The protein content was determined by Lowry method (Lowry et al., 1951).

All data are presented as means ± SD. Statistical data processing was performed with Microsoft Excel software using Student's *t*-test. The differences were considered significant if $p \leq 0.05$.

Results and discussion. The results of the study showed a 5-fold depletion of the hepatic tyrosine pool (fig. 1) with a simultaneous decrease in tyrosine aminotransferase activity more than by 2 times (fig. 2) under the conditions of low-protein ration in rats. These changes could be explained conclusions by the formation of endogenous tyrosine deficiency due to the alimentary lack of protein or an increased use of tyrosine by other tissues to form biologically active compounds, which precursor is tyrosine.

In particular, tyrosine either may be used in the adrenal medulla for the synthesis of catecholamines – dopamine, noradrenaline and adrenaline; or may serve as a precursor of a pigment melanin in melanocytes, and thyroid hormones – triiodothyronine and thyroxine – in the thyroid. (Syrovaya et al. 2014; Chernykh, 2013).

An established decrease in activity of tyrosine aminotransferase that catalyzes the first reaction of tyrosine transformation in liver tissue – transamination with α-ketoglutaric acid to form 4-hydroxysphenylpyruvate, is likely to be accompanied by a decrease in the content of the specified reaction product (Weiss, Refetoff 2016; Syrovaya et al. 2014).

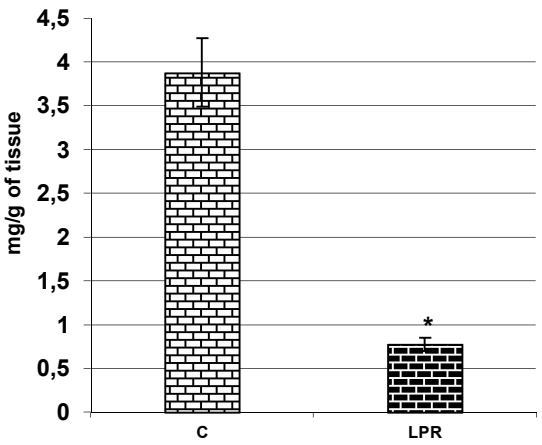

Fig. 1 The tyrosine content in the liver rats under the condition of alimentary protein deprivation

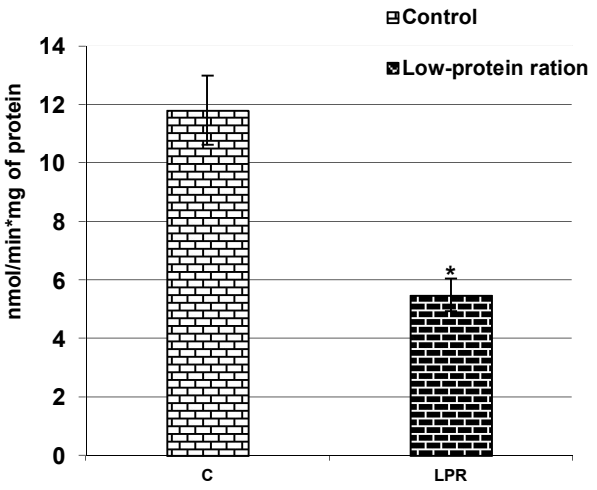

Fig. 2. The tyrosine aminotransferase activity in the rat liver mitochondrial fraction under the condition of alimentary protein deprivation

*Note (here and forwards): C – animals receiving complete semi-synthetic ration; LPR – animals receiving low-protein ration; * significantly different from the control, P≤0,05*

The formed 4-hydroxyphenylpyruvate may be further metabolized in two ways. The first is homogentisic with the formation of fumarate and acetoacetate, which are used as substrates in energy metabolism reactions. The other way is the tyrosine metabolism to 4-hydroxybenzoate – the precursor of he benzoyl ring of ubiquinone molecule (Antonenko et al., 2015; Donchenko et al., 2005).

4-hydroxyphenylpyruvate dioxygenase catalyzes the oxidation reaction of 4-hydroxyphenylpyruvate to homogentisic acid. This enzyme provides decarboxylation, hydroxylation of the aromatic ring, and migration of the 4-hydroxyphenylpyruvate side chain (Varela-López et al., 2016). The results of the present study showed the activity of 4-hydroxyphenylpyruvate dioxygenase remains at the control level under the conditions of alimentary protein deprivation (fig. 3).

Considering that fumarate and acetoacetate are the final products of the homogentistic pathway, the maintenance of the studied enzyme activity at the control level under the conditions of pronounced decrease in tyrosine content in liver tissue may be considered as a compensatory response aimed to maintain the energy metabolism. The formation of fumarate as a final product of the homogentistic pathway is an anaploretic reaction, which provides a replenishment of this Krebs cycle intermediate.

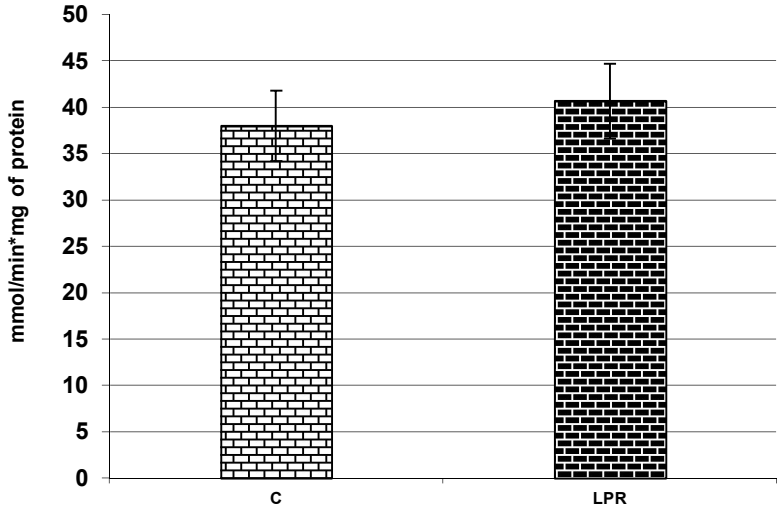

Fig. 3 The 4-hydroxyphenylpyruvate dioxygenase enzymatic activity in the rat liver mitochondrial fraction under the condition of alimentary protein deprivation

Previously, we have shown a decrease in succinate dehydrogenase activity (Voloshchuk et al., 2014) with the preservation of malate dehydrogenase reaction at the control level (Voloshchuk et al., 2015) under the conditions of protein deficiency.

Apparently, the replenishment of fumarate pool due to the reactions of homogentistic pathway is directed on the maintenance the functional activity of the Krebs cycle terminal enzymes, and respectively, on the preservation the ATP pool at the control level. At the same time, a 2-fold decrease in activity aldehyde dehydrogenase ALDH3A1, a key enzyme for the conversion of tyrosine to ubiquinone (Awad, 2018), was shown in conditions of the alimentary protein deficiency (fig. 4). The established fact explains the earlier results of our laboratory, which showed a 35% decrease in total ubiquinone content in mitochondria under the conditions of protein deficiency (Voloshchuk, Kopylchuk, 2015).

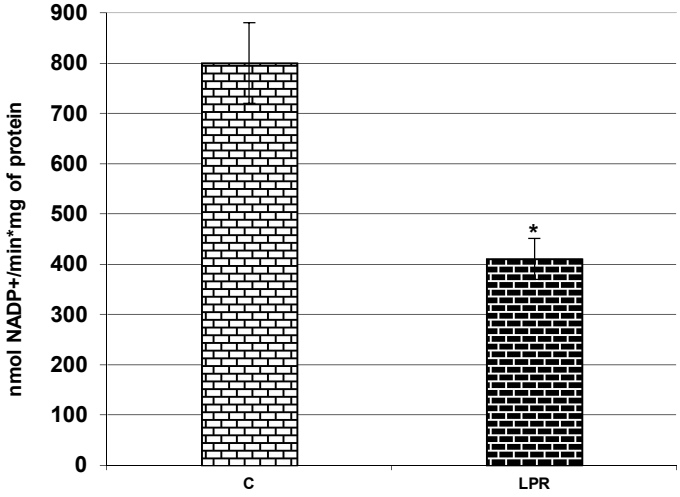

Fig. 4. The aldehyde dehydrogenase enzymatic activity of in the rat liver mitochondrial fraction under the condition of alimentary protein deprivation

Conclusions. Thus, under the conditions of alimentary protein deficiency, tyrosine in the liver is predominantly metabolized by the homogentisic pathway. It may be considered as a compensatory reaction, aimed at the maintenance of energy metabolism in simultaneous disturbance of the tyrosine use as a precursor for the ubiquinone synthesis.

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ОСОБЛИВОСТІ МЕТАБОЛІЗМУ ТИРОЗИНУ В ПЕЧІНЦІ ЩУРІВ ЗА УМОВИ АЛІМЕНТАРНОЇ НЕСТАЧІ ПРОТЕЇНУ

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У роботі досліджено вміст тирозину у печінці та активності ензимів його метаболізму: тирозинамінотрансферази, 4-гідроксифенілпірувat діоксигенази, альдегіддегідрогенази за умов аліментарної депривації протеїну. Експерименти проводилися на білих щурах масою тіла 100-150 г віком 2,5-3 місяці. Тварини були розділені на дві експериментальні групи: I – тварини, що отримували повноцінний напівсинтетичний раціон (К); II – тварини, що отримували низькопротеїновий раціон (НПР). З метою моделювання низькопротеїнової дієти тварини протягом 28 днів отримували раціон, що містив 4,7 % протеїну, 10 % жирів та 85,3 % вуглеводів, розрахований згідно з рекомендаціями American Institute of Nutrition. Визначення тирозину в депротеїнізованих 6% сульфосаліциловою кислотою екстрактах тканини печінки проводили на автоматичному аналізаторі амінокислот Т-339 (Мікротехнологія, Чехія). Активності ферментів визначали спектрофотометрично – тирозинамінотрансферази за кількістю 4-гідроксибензальдегіду, який має максимальну абсорбцію при 331 нм, 4-гідроксифенілпірувat діоксигенази – за інтенсивністю утворення забарвленого продукту при λ 336 нм, альдегіддегідрогеназну активність виміряли при довжині хвилі 340 нм. Встановлено, що за умов білкової недостатності у тканині печінки спостерігається 5-кратне виснаження пулу тирозину та зниження у понад 2 рази активності тирозинамінотрансферази, що каталізує утворення першого продукту перетворення тирозину – 4-гідроксифенілпірувату. Визначення активностей ключових ферментів двох можливих шляхів подальшого перетворення 4-гідроксифенілпірувату показало, що активність 4-гідроксифенілпірувat діоксигенази, фермента гомогентизинового шляху метаболізму тирозину, зберігається на рівні контролю, тоді як активність альдегіддегідрогенази, ключового ферменту шляху синтезу із 4-гідроксифенілпірувату бензойного кільця молекули убіхінону, знижується вдвічі. Отримані результати дозволяють зробити висновок, що за умов аліментарної нестачі протеїну тирозин у печінці переважно метаболізується гомогентизиновим шляхом, що можна розглядати як компенсаторну реакцію, спрямовану на підтримання енергетичного метаболізму, при одночасному порушенні використанні тирозину як попередника у синтезі убіхінону.

Ключові слова: аліментарна депривація протеїну, печінка, мітохондрії, тирозин, тирозинамінотрансфераза, 4-гідроксифенілпірувat діоксигеназа, альдегіддегідрогеназа

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