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Vitabolomics. New direction in the vitaminilogy

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Abstract

Modern studies of enzymovitamins are devoted to the study of the biochemical functions of their metabolically active forms. At the same time, it is not taken into account that in cells and in some cases in one compartment of the cell both the vitamin itself and its anabolites and catabolites co-exist together. It seems to us necessary to study the biochemical effect of the combined action of all the metabolites of a particular vitamin that present in the cells. We propose to name such a complex of all formed metabolites of individual vitamins as the vitabolome. Our studies, conducted with thiamine, riboflavin, and pantothenic vitabolomes, have demonstrated that in some cases the complexes of metabolites of these vitamins have regulatory properties that are fundamentally different from those of the corresponding vitamins and coenzymes. Thus, thiamine vitabolome significantly mitigates the activating action of thiamine and TPP on the activity of the pyruvate dehydrogenase complex in tissues and prevents the inhibitory effect of thiochrome on this multienzyme complex. Even more significant is the fact that thiamine vitabolome has a regulatory effect on certain enzymes, unlike thiamin or its metabolites alone. Studying the effect of individual metabolites of pantothenic acid and their complex on acetylation activity in tissues, we found that individual metabolites in physiological concentrations do not affect the recorded index, while pantothenic vitabolome exerted a pronounced effect, the direction of which depends on the terms after its introduction. Riboflavin vitabolome significantly activated succinate dehydrogenase, while riboflavin, FMN and FAD did not have such effect .

Аннотация

Современные исследования энзимовитаминов посвящены изучению биохимических функций их метаболически активных форм. В то же время не учитывается то обстоятельство, что в клетках, а в ряде случаев в одном компартменте клетки, сосуществуют как сам витамин, так и его анаболиты и катаболиты. Нам представляется необходимым изучение биохимического эффекта совместного действия всех присутствующих в клетках метаболитов того или иного витамина. Такой комплекс всех образующихся метаболитов отдельных витаминов мы предлагаем назвать витаболомом. Нашими исследованиями, проведенными с тиаминовым, рибофлавиновым, и пантотеновым витаболомами, продемонстрировали, что в некоторых случаях комплексы метаболитов выше указанных витаминов обладают регуляторными свойствами, принципиально отличающимися от таковых соответствующих витаминов и коферментов. Так, тиаминовый витаболом существенно смягчает активирующее действие тиамина и ТПФ на активность пируватдегидрогеназного комплекса в тканях и предотвращает ингибирующее действие тиохрома на этот мультиэнзимный комплекс. Еще более существенным является то, что тиаминовый витаболом оказывает регулирующее действие на некоторые ферменты, на которые ни тиамин, ни его метаболиты в отдельности не влияют. При изучении воздействия отдельных метаболитов пантотеновой кислоты и их комплекса на ацетилирующую активность в тканях нами установлено, что отдельные метаболиты в физиологических концентрациях не влияют на регистрируемый показатель, в то время как пантотеновый витаболом оказывал выраженное действие, направленность которого зависит от сроков после его введения. Рибофлавиновый витаболом существенно активировал сукцинатдегидрогеназу, в то время как рибофлавин, ФМН и ФАД таким эффектом не обладали

Keywords: Laser Vitabolomics, Vitamins, Coenzymes, Catabolites.

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1. Introduction

In contemporary vitaminology, the predominant notion claims that the single biochemical function of an enzyme-vitamin appears to be the function of coenzyme for a corresponding enzyme.

However, during last three decades, a significant number of researches, disproving this notion, have been published. Their results indicate that the enzyme-vitamins and their metabolites have their own biochemical functions, which have nothing to do with the coenzyme activity [2-7]. In our laboratory, biochemical functions of catabolites of thiamine, riboflavin, pantothenic acid, nicotinic acid, pyridoxine and ascorbic acid have been studied [8-10].

In the process of investigation the functions of enzyme-vitamins and their catabolites in the body, one should keep in mind that the vitamin itself, its coenzymes and other metabolites are present simultaneously in the tissues. Therefore, the objective of this study was to study the possibility of the cooperative participation of metabolites of vitamins, found in tissues, in the regulation of various multistage biochemical processes.

Actually every vitamin, this applies in particular to coenzyme vitamins, is contained in cells in the form of certain metabolites through which it can realize its functions. For example, vitabolome of vitamin B1, thiamine, includes thiamine monophosphate, thiamine diphosphate, thiamine triphosphate, and also simple and mixed disulfides, recently found and studied adenylated thiamine triphosphate. In addition to these compounds, the cells contain a certain amount of other thiamine metabolites. The biochemical activity of thiochrome is intensively studied in our laboratory. Vitamin B_2 realizes its functions through the co-enzymes of FAD and FMN, however, the cells also contain the original form of the vitamin - riboflavin itself, in addition, other riboflavin catabolites are present in the cells, for example, lumichrome and lumiflavine. Earlier it was thought that these are inert compounds, which are unnecessary ballast in cells. Their functions are also investigated in our laboratory. Vitamin B_5, in a large number of publications, known as vitamin B_3, deciphering as pantothenic acid, has a basic coenzyme form called coenzyme A, which function of the transporting acyl groups to energy-significant enzyme cycles, for example, to the Krebs cycle, has long been known. But in the cells, apart from pantothenic acid, there are also other derivatives of pantothenic acid, and compounds which are structural units or metabolic products of coenzyme A. These are phosphopantothenate, β-alanine, sodium panthoate, pantethine A and other compounds. Other systems of metabolites of vitamins also exist in cells. For example, there is ascorbic acid metabolites system comprising, in fact, ascorbic acid, mono- and dehydroascorbic acids, diketogulonic, threonic, oxalic acids. There are publications devoted to other metabolites of vitamin C, for example, L-xylonic acid and its lactone, L-lyxonic and L-erythroaskorbic acids. Publications on the combined effect of vitamin C metabolites on cellular metabolism are limited, and this concept is not well developed and supported by all.

Professor S.A.Petrov proposed the term "vitabolome" by analogy with metabolone. In our therminology, vitabolome is a combination of a given vitamin and its metabolic-catabolite forms, which form a specific system in cells and are in certain ratios corresponding to a certain level of cellular metabolism. Their concentration and ratios in cells determine, regulate and limit the functional activity of cellular processes. In this article, we made an attempt to investigate the vitabolomes of vitamins B_1, B_2 and B_5.

2. Material and Methods

The experiments were conducted at the Department of Biochemistry of Odessa I. I. Mechnikov National University. Albino mongrel rats of different ages were used. As a biomaterial, extracts of organs lacking cell membranes were used. The use of such an object as tissue extracts devoid of cell membranes is caused by the necessity of having all components of the cell in the environment, since the realization of the functions of vitamin metabolites can be carried out in different cell compartments. Before determining the biochemical parameters, the membrane-free extracts of the organs were introduced into the incubation medium and all the metabolites were added in the ratios present in the tissues. In particular: metabolites of vitamin B_1: thiamine, thiamine pyrophosphate (TPP), thiochrome, 4-methyl-5-β-hydroxyethylthiazole, a mixture of these metabolites, called vitabolome of vitamin B_1; metabolites of vitamin B_2: riboflavin, FMN, FAD, lumichrome (LC), a mixture of these metabolites called vitamin's B_2 vitabolome; metabolites of vitamin B_5: phosphopantothenate, β-alanine, calcium α-pantothenate, sodium pantoate, pantetin A, CoA, a mixture of these metabolites, which was called vitamin's B_5 vitabolome. Activity of the pyruvate dehydrogenase complex and succinate dehydrogenase was defined by Potassium ferricyanide method; the activity of CoA transacetylases was defined by the speed of acetylation [12], the activity of acetylcholinesterase has been measured according to the standard protocol by colorimetric method of acetylcholine hydrochloride hydrolysis with the formation of choline and acetic acid, which caused a change in pH [13], and the activity of transketolase was defined by the photometric method for the intensity of the formation of a colored complex of pentose with orcine [14]. Earlier, we determined the average concentrations of vitamin metabolites in some organs. Therefore, in the study of vitabolomes, we used precisely these concentrations and ratios of the metabolites present in tissues in situ. As we established earlier, some catabolites of vitamins, even in insignificant concentrations compared to the vitamin itself, have a marked effect on the

Table 1 | The content of thiamine (μg/g), riboflavin (μg/g), pantothenic acid (nmol/g) and their main metabolites in the liver of white rats (n=8). * - significant in relation to the content of the corresponding vitamin, $p \leq 0.05$. Содержание тиамина (мкг / г), рибофлавина (мкг / г), пантотеновой кислоты (нмоль / г) и их основных метаболитов в печени белых крыс (n = 8). * - достоверно по отношению к содержанию соответствующего витамина, $p \leq 0,05$.

Thiamine and its metabolites (μg/g)		Riboflavin and its metabolites (μg/g)		Pantothenic acid and its metabolites (nmol//g)	
TPP+TMP	1,5±0,2*	FAD	124,0±2,0*	KoA	165,0±17,0*
Thiamine	0,4±0,1	FMN	6,2±0,7*	Panthetine	2,0±0,3*
Thiochrome	0,4±0,1	Riboflavin	0,8±0,1	Pantothenate	12,1±1,3
4-methyl-5-β-hydroxyethyl-thiazole	0,1±0,1*	Lumichrome	0,3±0,1*	Pantoate	0,8±0,1*

activity of certain enzymes. Everything related to laboratory animals (keeping, conducting experiments) were with strict compliance to international rules: «Guide for the Care and Use of Laboratory Animals». All obtained data was processed statistically. Values are reported as means ± SE. All data were analyzed using one-way ANOVA (Biostat). Statistical significance was considered to be $p \leq 0.05$ [16].

3. Results and discussion

In At the beginning of our research, we determined the average content of thiamine, riboflavin, pantothenic acid and their main metabolites in the liver of Albino mongrel rats. These data are presented in Table 1.

As it can be seen from the results presented in table 1, the prevailing metabolic form of all the studied vitamins is the coenzyme form. However, small amounts of free vitamins and even smaller amounts of their catabolites are present in the liver.

Therefore, in further studies of the effects of vitabolome, we used a mixture of catabolites in the concentrations and ratios indicated in Table 1, which are present in vivo in the liver.

During investigation of the regulatory properties of thiamine's vitabolome, the following patterns have been detected. In the liver of white rats, the sum of thiamine metabolites significantly mitigate the activating action of thiamine and thiamine pyrophosphate (TPP) on the activity of the pyruvate dehydrogenase complex (PDC) and prevents the inhibitory effect of thiochrome on it (Figure 1).

During the study of the activity of transketolase in the presence of thiamine, its metabolites and vitabolome, it was detected that the activity of transketolase is different in different organs and tissues (blood, liver, brain, adrenals). This activity is maximal in the brain and minimal in the liver. However, the nature of the effect of thiamine metabolites (thiamine and TPP) on the activity of transketolase is practically the same. Thiamine vitabolome also stimulated the activity of this enzyme, and this effect was a bit greater than in the case of TPP (Figure 2).

In the course of the study of the regulatory role of pantothenic acid, its metabolites and pantothenic acid's vitabolome on the transacetylating activity in the tissues of white rats (Figure 3), we found out that none of the metabolites of pantothenate had a significant effect on transacetylase activity in the kidneys and heart. Pantothenic acid's vitabolome also proved to be ineffective in these organs. In the liver, activating action was demonstrated by β -alanine, and in the brain by panthetine. The CoA effect was unexpected for us. The addition of CoA into the incubation medium reduced the investigated index in the liver by 10%. Pantothenic acid's vitabolome in the liver led to the decrease in transacetylase activity. In other organs studied, pantothenic acid's vitabolome did not affect the investigated

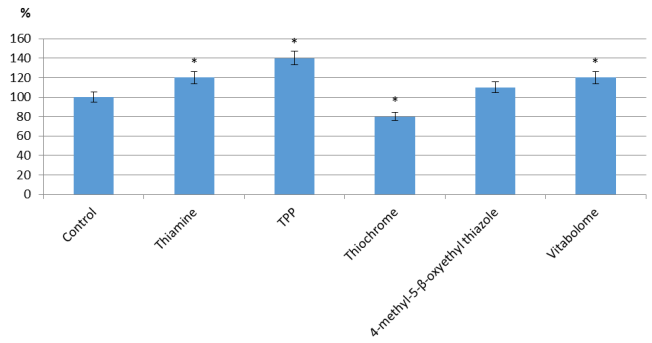

Figure 1 | Influence of individual thiamine metabolites and thiamine's vitabolome on the activity of the pyruvate dehydrogenase complex in the liver of white rats (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние отдельных метаболитов тиамина и витаболома тиамина на активность пируватдегидрогеназного комплекса в печени белых крыс (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем

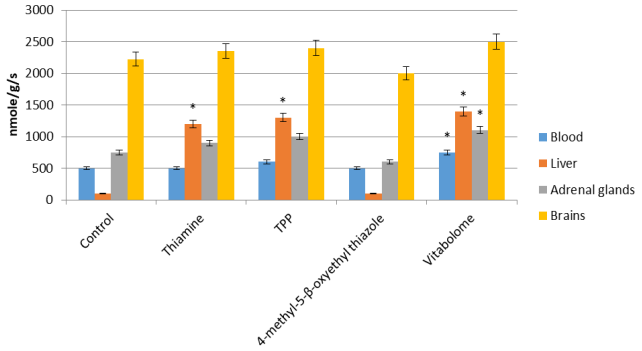

Figure 2 | Influence of pantothenate and its metabolites and pantothenic acid's vitabolome on transacetylase activity in tissues of white rats (% of control) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние пантотената и его метаболитов и витаболома пантотеновой кислоты на трансацетилазную активность в тканях белых крыс (% от контроля) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

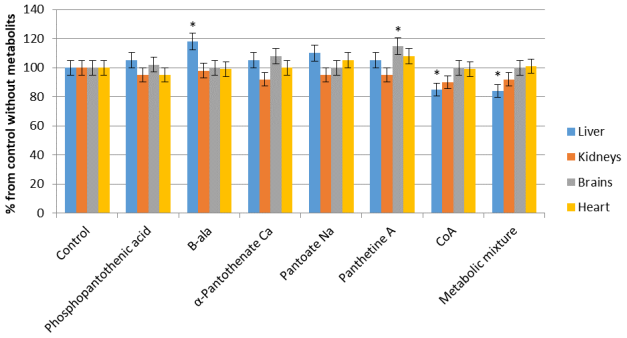

Figure 3 | Influence of pantothenate and its metabolites and pantothenic acid's vitabolome on transacetylase activity in tissues of white rats (% of control) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние пантотената и его метаболитов и витаболома пантотеновой кислоты на трансацетилазную активность в тканях белых крыс (% от контроля) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

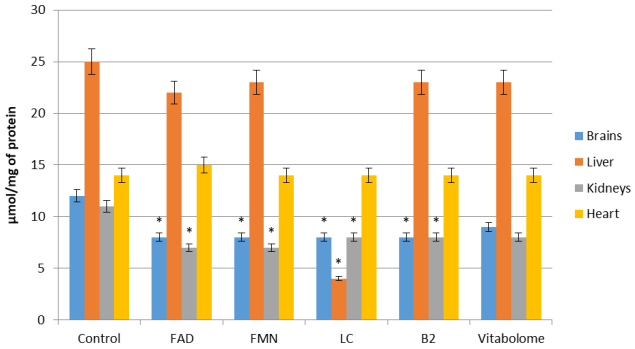

Figure 5 | Influence of riboflavin, its metabolites and riboflavin'svitabolome on the activity of PDC in the organs of mature (10-12 months) rats (μM / mg protein) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома рибофлавина на активность пируватдегидрогеназного комплекса в органах зрелых (10-12 месяцев) крыс (мкМ / мг белка) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

index. To explain the effects, series of additional studies are required.

The most prominent features of riboflavin's vitabolome appeared in studies of the regulation of the activity of the multi-enzyme pyruvate dehydrogenase complex. The effect of riboflavin and its metabolites on the activity of the multienzyme pyruvate dehydrogenase complex depends on the age of animals used for the experiments. In particular, in the organs of old rats (22-24 months), neither riboflavin nor its metabolites and vitabolome had an effect on the activity of the pyruvate dehydrogenase complex (PDC) (Figure 4).

In the group of mature rats, it was detected that neither riboflavin, nor its metabolites (except lumichrome) and riboflavin vitabolome had any effect on the PDC activity in liver and heart. All studied metabolites of riboflavin reduce the activity of the PDC in brain and kidneys, while the vitabolome to some extent mitigated this inhibitory effect (Figure 5).

In general, similar pattern was observed in the organs of young (5-6 months) rats (Figure 6). All metabolites of riboflavin reduce the activity of PDC in brain, while riboflavinic vitabolome returned the investigated index to the control values. Only FAD and lumichrome reduced the activity of PDC in liver, and the vitabolome had no protective effect as it was shown in previous organs. FMN significantly decreased the activity of PDC in kidneys. The riboflavinic vitabolome approximated the investigated index to the control values.

Besides, we conducted there the search on effects of riboflavin, its metabolites and vitabolome on the activity of succinate dehydrogenase (SDH) in organs of white rats. In old animals (22-24 months), the content of the riboflavin's metabolites and its vitabolome varied in different organs (Figure 7). FMN and LC showed the inhibitory action on SDH in heart. Vitabolome also reduced this effect. In brain, the addition of FMN into the medium led to the activation

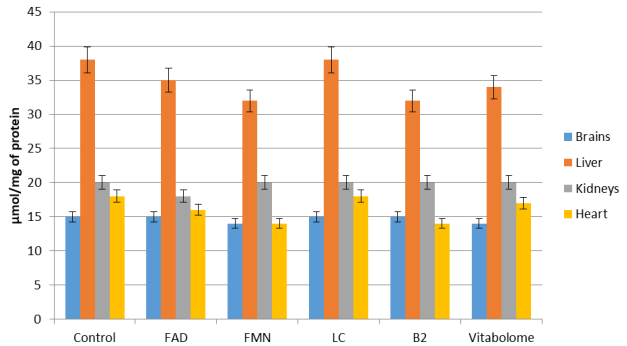

Figure 4 | Influence of riboflavin, its metabolites and riboflavin's vitabolome on the activity of PDC in the organs of old (22-24 months) rats (μM / mg protein) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома рибофлавина на активность пируватдегидрогеназного комплекса в органах старых (22-24 месяца) крыс (мкМ / мг белка) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

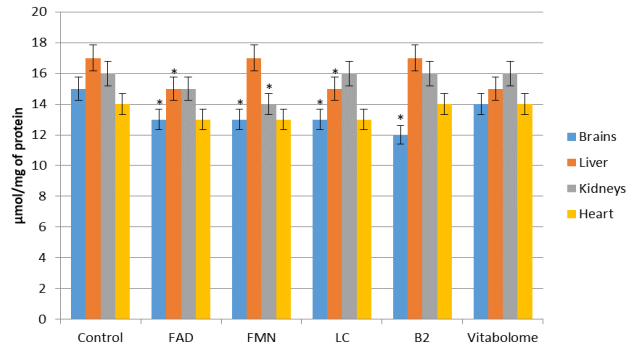

Figure 6 | Influence of riboflavin, its metabolites and riboflavin's vitabolome on the activity of PDC in the organs of young (5-6 months) rats (μM / mg protein)(n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома рибофлавина на активность пируватдегидрогеназного комплекса в органах молодых (5-6 месяцев) крыс (мкМ / мг белка) (n = 8).Примечания: * - $p \leq 0,05$ по сравнению с конт .

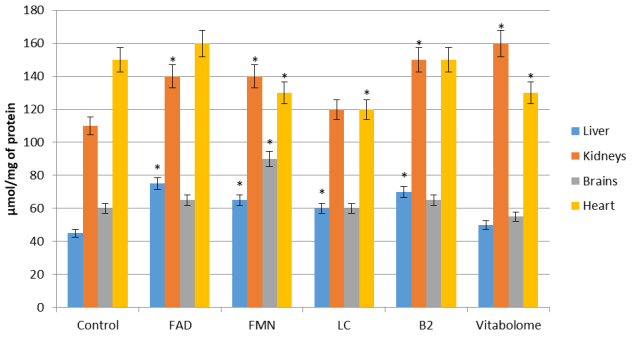

Figure 7 | Influence of riboflavin, its metabolites and its vitabolome on the activity of SDH in the organs of old (22-24 months) rats (nmol / mg protein) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и его витаболома на активность СДГ в органах старых (22-24 месяца) крыс (нмоль / мг белка) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

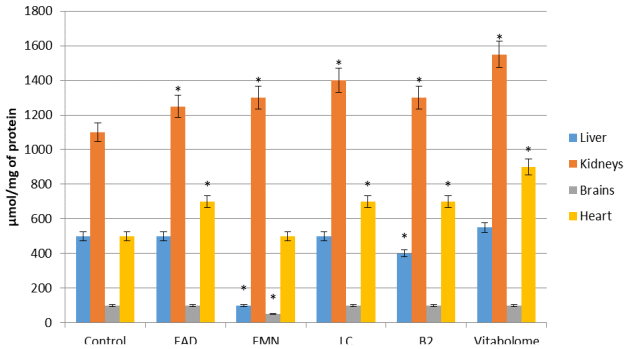

Figure 9 | The effect of riboflavin, its metabolites and vitabolome on the activity of SDH in the organs of young (5-6 months) rats (nmol/mg protein) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома на активность СДГ в органах молодых (5-6 месяцев) крыс (нмоль / мг белка) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем.

of SDH. Vitabolome returned this pattern to the control values. The activating action of riboflavin, FMN, LC and FAD was significantly reduced in the presence of the vitabolome in the liver.

Riboflavin had an activating action on activity of SDG but FMN and LC decrease the activity of SDG in the liver of mature rats (10-12 months) (Figure 8). FAD increase the activity of SDG, but riboflavin and LC decrease the activity of SDG in the heart. All of the metabolites of riboflavin decrease the activity of SDG in the brain. In all these organs, riboflavin's vitabolome significantly changed these effects.

In the heart and kidneys of young animals (5-6 months) riboflavinic vitabolome activated SDH greater than individual metabolites. Riboflavin and FMN decreased the activity of SDG in liver,and the effect of the vitabolome does not differ from the effect of FAD on activity of SDG (Figure 9).

Discussion of the results obtained, while studying the effect of vitabolome on enzymes that do not contain vitamins' metabolites as coenzymes, deserve special attention. Acetylcholinesterase (AChE) was chosen as such an enzyme. The most noticeable fact is that riboflavin and its metabolites and vitabolome had an activating effect on the activity of acetylcholine esterase in the study of activity in the liver of old (22-24 months) rats. In the liver of young animals only riboflavinic vitabolome significantly increased the activity of AChE (Figure 10).

Thus, our studies have shown the need to take into account the simultaneous presence of various metabolites of vitamins in tissues when examining their effects on various biochemical processes. Our research has demonstrated that in a significant number of cases the action of the vitabolomes of various vitamins differs from the action of both coenzyme forms and catabolites.

The mechanisms of such action can be realized both at the level of individual enzymes, and in competition for the corresponding proteins

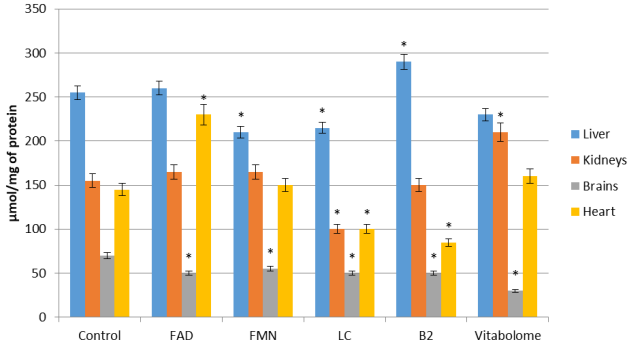

Figure 8 | Influence of riboflavin, its metabolites and vitabolome on the activity of SDH in the organs of mature (10-12 months) rats (nmol / mg protein) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома на активность СДГ в органах зрелых (10-12 месяцев) крыс (нмоль / мг белка) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем .

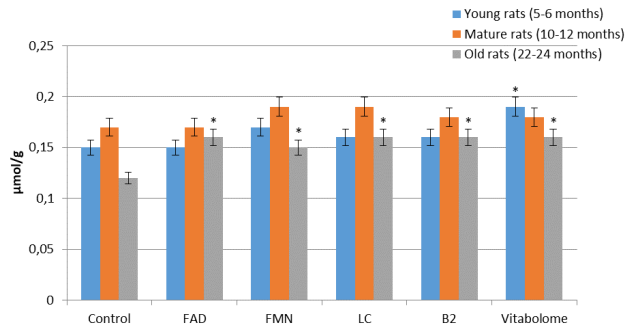

Figure 10 | Influence of riboflavin, its metabolites and vitabolome on AChE activity in liver of young (5-6 months), mature (10-12 months) and old (22-24 months) rats (µmol/g) (n=8). Notes: * - $p \leq 0.05$ in comparison with the control. Влияние рибофлавина, его метаболитов и витаболома на активность ацетилхолинэстеразы в печени молодых (5-6 месяцев), зрелых (10-12 месяцев) и старых (22-24 месяцев) крыс (мкмоль / г) (n = 8). Примечания: * - $p \leq 0,05$ по сравнению с контролем:

5. Concluding Remarks

1. The presence of the vitabolomes of the corresponding vitamins in tissues leads to modulating effects on the corresponding vitamin-dependent enzymes, which is manifested in a decrease in the activating effect and a weakening of the inhibitory action of catabolites.
2. The effectiveness of the action of the vitabolomes significantly depends on the age of the experimental animals.

Заключение

1. Наличие в тканях витаболомов соответствующих витаминов приводит к модулирующим эффектам в отношении соответствующих витаминзависимых ферментов, что выражается в снижении активирующих эффектов витаминов и ослаблении ингибиторного действия катаболитов.
2. Эффективность действия витаболомов существенно зависит от возраста экспериментальных животных

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