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Thiaminase process is present in the brain of mammals

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ABSTRACT

The presence of the thiaminase process in the brain of rabbits and bulls has been established. Most intensively this process takes place in the pituitary gland. Production of adrenocorticotropic hormone is significantly reduced as a result of the formation of 4-methyl-5β-oxyethylthiazole in this part of the brain.

Аннотация

Установлено наличие тиаминазного процесса в мозге кроликов и быков. Наиболее интенсивно этот процесс происходит в гипофизе. Продукция адренокортикотропного гормона значительно снижается в результате образования 4-метил-5β-оксиэтилтиазола в этой части мозга.

Keywords: Thiaminase, brain, pituitary gland, 4-methyl-5β-oxyethylthiazole, ACTH, TSH

1. Introduction

Thiaminase is an enzyme that divides thiamine into two parts-4-methyl-5β-oxyethylthiazole and 2,5-dimethyl-4-aminopyrimidine. There are two forms of thiaminase - thiaminase I and thiaminase II.

Thiaminase I catalyzes the cleavage of thiamine with subsequent adherence to the pyrimidine fragment of the nitrogenous base. Tiaminase II carries out the hydrolysis of thiamine into the two above-mentioned components [1].

The presence of thiaminase I is proven in tissues of fish, intestinal cavities, mumps, and some microorganisms [1]. Thiaminase II is present exclusively in microorganisms, in particular *Bac. alvei, Bac. brevis, Bac. firmus, Bac. subtilis* and some others [2].

Until recently, it was generally accepted that in human and other mammals, the thiamine decompositiondown to the thiazole and pyrimidine components was carried out exclusively in the intestines at the expense of its microflora, in particular *Bac. aneurinolyticus* [3], *Clostridium thiaminolyticum* [4], *Bacillus thiaminolyticus* [5] and some others.

However, since the 1960s of the last century, there were single studies that showed the presence of 4-methyl-5β-oxyethylthiazole in the urine of sterile animals [6], which proves the possibility of the thiaminase process existencedirectly in the tissues. In our laboratory, a significant increase in the level of 4-methyl-5β-oxyethylthiazole in the brain of rats after parenteral administration of 14C-thiamine [7] was demonstrated. Linkage of the thiazole with nervous system proteins has been demonstrated in a recently published work [8].

Studies of thiaminase pathways are considered in the works [9-11], however, studies on the functioning of thiaminase and its physiological significance in the brain do not exist.

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Table 1 | The content of thiamine and its metabolites in blood taken from the jugular vein, *venae jugularis externa*, after injection of thiamine into the carotid artery (μg/ml of blood) n=8. * – the difference with the control group is significant (p≤0.05). Содержание тиамина и его метаболитов в крови, взятой из яремной вены, *venae jugularis externa*, после введения тиамина в сонную артерию (мкг / мл крови) n = 8. * - различия с контрольной группой достоверны (p≤0,05).

Parameter	Control	1 minute	2 minutes	5 minutes	10 minutes
T-S-S-T	0.070±0.006	0.095±0.010	0.120±0.011*	0.155±0.017*	0.160±0.014*
TPP	0.355±0.037	0.470±0.048	0.595±0.061*	0.590±0.058*	0.590±0.062*
Thiamine	0.795±0.080	0.510±0.040*	0.400±0.038*	0.405±0.041*	0.410±0.039*
4-methyl-5β-oxyethylthiazole	0.030±0.003	0.055±0.004*	0.085±0.007*	0.085±0.009*	0.090±0.009*

2. Material and Methods

All studies were carried out on rabbits of 3-3.5 years old and the brain of bulls. The norms of the European convention on carrying out experiments with vertebrates have been adhered to the current research.

The content of thiamine and TPP was measured by fluorimetric method [12]. The content of 4-methyl-5β-oxyethylthiazole was determined after separation of thiamine and its metabolites using spectrophotometry after column chromatography at 253 nm [13].In this work, DEAE -cellulose was used for the chromatographic separation of thiamine metabolites, since the stability constants of their complexes, which are formed, and, therefore, their adsorption properties at certain pH differ very significantly.

Thiamine disulfide was determined by the fluorimetric method [1].

Determination of adrenocorticotropic (ACTH) and thyroid stimulating (TT) hormones was carried out by immunoassay analysis [14, 15].

The obtained results are processed by methods of variation statistics. The normality of distribution is rechecked using [16]. Values are reported as means ± SE. For further analysis, the Student's t-test was used, or its modification - Newman–Keuls method by Glantz S., 1999 [17]. Statistical significance was considered to be $p \leq 0.05$.

3. Results

We started research on whether the thiaminase process in the brain was present. For this, 1 mg of thiamine was injected into the carotid artery (arteria carotis externa) of rabbit and in 1, 2, 5, and 10 minutes blood samples from the jugular vein (venae jugularis externa) were collected and the content of thiamine, TPP, thiamine disulphide (TSST) and 4 -methyl-5β-oxyethylthiazole were determined.

The results of this study are presented in Table 1.

The obtained results indicate that injections of thiamine into the carotid artery arteria carotis externa lead to its rather rapid metabolism in the brain tissues. So, 1 minute after injection, a significant increase in the content of 4-methyl-5β-oxyethylthiazole was observed due to a decrease in the thiamine content, indicating a rapid decay in the brain. Two minutes after injection, the level of 4-methyl-5β-oxyethylthiazole continued to increase, the amount of TPP and T-S-S-T also increased, while the thiamine level continued to decrease. In 5 and 10 minutes, further significant changes in the level of thiamine and its metabolites in the blood were not observed.

Thus, this part of the research shows that after thiamine entrance into the brain, the transformation of its part first occurs due to thiaminasedecomposition. Later, the remnants of free thiamine are converted into coenzyme (TPP) and into the reserve form T-S-S-T.

The next step of our research was to find out in which part of the brain thiaminase process is most intense. These data are shown in Tables 2 and 3.

To solve this problem, the following parts of the brain of the bull were separated: cortex, pons, cerebellum, pituitary gland, thalamus + hypothalamus and corpus callosum. Each department was perfused with glucose solution with thiamine at a certain concentration for 5 minutes. The content of thiamine, TPP and 4-methyl-5β-oxyethylthiazole was determined before and after perfusion.

The results presented in Table 2 indicate that the highest content of free thiamine was observed in the corpus callosum.

In all other investigated brain departments, except for cortex, it does not differ significantly. In the cortex, this value was more than twice as low. The TPP content was the largest in the corpus callosum, it was slightly lower in the pons, the pituitary gland and thalamus + hypothalamus. The smallest one was registered in the cortex. The level of 4-methyl-5β-oxyethylthiazole was highest in the corpus callosum, and the lowest in thalamus + hypothalamus.

Perfusion with thiamine and glucose increased the level of free thiamine in the corpus callosum, while the level of TPP significantly increased in all of the studied brain departments, but the greatest effect was established in the pons and thalamus + hypothalamus. Under these conditions, the level of 4-methyl-5β-oxyethylthiazole was significantly increased in the pituitary gland and cortex. In the cerebellum this increase was less pronounced.

Thus, relatively high doses of thiamine resulted in its accumulation in the unchanged form in the corpus callosum. Subsequently, its part was converted into the

Table 2 | The content of thiamine, TPP and 4-methyl-5β-oxyethylthiazole in the brain parts of the bull after perfusion with thiamine (1 mg / ml) solution (mg/g of tissue) n=8. * – the difference with the baseline group is significant (p≤0.05). Содержание тиамина, ТПП и 4-метил-5β-оксиэтилтиазола в отделах головного мозга быка после перфузии раствором тиамина (1 мг / мл) (мг / г ткани) n = 8. * - различия с соответствующим контролем достоверны (p≤0,05).

Brain department	Thiamine		TPP		4-methyl-5β-oxyethylthiazole	
	baseline	increase	baseline	increase	baseline	increase
Cortex	0.36±0.02	2.12±0.04*	0.66±0.05	5.54±0.23*	0.64±0.08	4.46±0.05*
Pons	0.78±0.02	2.12±0.07*	0.92±0.04	4.46±0.20*	0.62±0.06	1.40±0.04*
Cerebellum	0.78±0.02	1.76±0.56*	0.80±0.17	1.82±0.45*	0.42±0.07	1.32±0.08*
Pituitary gland	0.64±0.07	2.64±0.24*	0.90±0.06	7.18±0.31*	0.58±0.06	8.62±0.13*
Thalamus + hypo-	0.72±0.04	2.78±0.18*	0.92±0.14	4.16±0.13*	0.14±0.02	0.50±0.03*
Corpus callosum	0.88±0.02	6.24±0.10*	1.04±0.11	6.12±0.04*	0.86±0.02	1.20±0.11*

Table 3 | Increase of thiamine, TPP and 4-methyl-5β-oxyethylthiazole content in the bovine brain sections with perfusion of thiamine (0.1 mg/ml) (μg/g of tissue) n=8. Увеличение содержания тиамина, ТПП и 4-метил-5β-оксиэтилтиазола в отделах головного мозга крупного рогатого скота при перфузии тиамина (0,1 мг / мл) (мкг / г ткани) n = 8

Brain department	Thiamine	TPP	4-methyl-5β-oxyethylthiazole
Cortex	1.16±0.05	1.98±0.09	0.92±0.05
Pons	1.10±0.03	2.10±0.08	0.86±0.02
Cerebellum	0.96±0.05	1.76±0.05	0.84±0.04
Pituitary gland	1.06±0.04	1.38±0.24	1.36±0.20
Thalamus + hypothala-mus	1.06±0.04	1.98±0.19	0.60±0.10
Corpus callosum	2.16±0.17	1.82±0.05	0.68±0.12

Table 4 | Determination of the concentration of ACTH and TSH in blood taken from the jugular vein after administration of 4-methyl-5β-oxyethylthiazole solution in the carotid artery at a concentration of 1 mg/ml (μM/ml of blood) n=8. * – the difference with the control group is significant (p≤0.05). Определение концентрации АКТГ и ТТГ в крови, взятой из яремной вены, после введения раствора 4-метил-5β-оксиэтилтиазола в сонную артерию в концентрации 1 мг / мл (мкМ / мл крови) n = 8. * - различия с контрольной группой достоверны (p≤0,05).

Time after ad-	ACTH	TSH
Control	15.0±0.9	6.0±0.5
5 minutes	10.1±0.8*	5.0±0.5
10 minutes	8.2±0.7*	4.2±0.4*
15 minutes	6.1±0.7*	3.5±0.4*
20 minutes	4.0±0.3*	1.0±0.2*

major coenzyme form of TPP in all parts of the brain, but to a greater extent in the pons and thalamus + hypothalamus. Intensive thiamine decompositionby thiaminase occurred in the pituitary gland and cortex.

A decrease in the thiamine dose to 0.1 mg / ml resulted in the following effects (Table 3).

The results presented in Table 3 indicate that with the use of 0.1 mg/kg of thiamine, the highest amount of free thiamine was present in the corpus callosum, as with after the use of 1 mg / ml. In other parts of the brain its contents did not differ significantly. The content of TPP in all studied brain departments, except for the pituitary gland, was almost similar. In the pituitary glandit was the smallest. As for 4-methyl-5β-oxyethylthiazole, its content was significantly higher in the pituitary gland than in other parts of the brain.

Thus, this part of our study suggests that thiaminase pathway of thiamine decomposition takes place in the brain, resulting in the formation of 4-methyl-5β-oxyethylthiazole. This processis most intensive in the pituitary gland.

To find out the possible specific physiological function of 4-methyl-5β-oxyethylthiazole in the pituitary gland, we conducted the next series of studies.

We injected 4-methyl-5β-oxyethylthiazole solution into the carotid artery of the rabbit, and after 5, 10, 15 and 20 minutes, the blood from the jugular vein was taken out and the content of ACTH and TSH was measured. The control included administration of saline solution. Data for this series are presented in Table 4.

As can be seen from the data presented in Table 4, administration of 4-methyl-5β-oxyethylthiazole in the carotid artery at a concentration of 1 mg/ml resulted in a significant decrease in both ACTH and TSH levels in 20 minutes after administration. The level of ACTH was reduced by almost 4-fold, and SH - 6 times in 20 minutes after injection.

When the concentration of 4-methyl-5β-oxyethylthiazole was reduced by 100 times to a concentration of 10 μg/ml, which corresponds to the physiological concentration in the blood, we obtained the following results (Table 5).

The results presented in Table 5 indicate that physiological concentrations of 4-methyl-5β-oxyethylthiazole can reduce the level of ACTH, while they do not affect the level of TSH.

Table 5 | Determination of the concentration of ACTH and TSH in blood taken from the jugular vein after the introduction of 4-methyl-5β-oxyethylthiazole solution in a carotid artery at a concentration of 10 μg/ml (μM/ml of blood) n=8. * – the difference with the control group is significant (p≤0.05). Определение концентрации АКТГ и ТТГ в крови, взятой из яремной вены, после введения раствора 4-метил-5β-оксиэтилтиазола в сонную артерию в концентрации 10 мкг / мл (мкМ / мл крови) n = 8. * - различия с контрольной группой достоверны (p≤0,05).

Time after administration	ACTH	TSH
Control	15.0±1.1	11.0±1.2
5 minutes	14.0±0.9	11.0±1.0
10 minutes	13.6±1.3	11.0±1.0
15 minutes	11.1±0.9*	10.2±1.1
20 minutes	12.0±0.9*	10.1±1.2

The obtained data confirm the presence of enzymatic degradation of thiamine in the brain. The discovery of this 4-methyl-5β-oxyethylthiazoledegradation product in the body was reported in 1969 [6]. It is important to note that this experiment was conducted on sterile animals, which excluded the participation of microorganisms in this process. In our laboratory, this fact has been confirmed [7].

In 2015, a large international team of researchers reported the interaction of thiazole and its derivatives with proteins of the nervous system [8].

In our study, it was established that the formation of this product of thiaminase reaction in the brain occurs in its different parts, but the most intensively this process is conducted in the pituitary gland. According to our data, the physiological role of this process includes inhibition of adrenocorticotropic hormone adenohypophysis.

This fact agrees well with the observations of several authors on the influence of thiamine on some processes with the regulatory participation of adrenaline, in particular carbohydrates metabolism [18].

In the future, we are going to isolate and purify thiaminase using first classical methods, such as salting out, dialysis, column chromatography on different carriers and electrophoresis to assess the degree of purification of the enzyme.

4. Concluding Remarks

Thus, the materials presented in this study indicate the existence of hormone-mediated non-enzymatic function of the product of the thiaminase process of 4-methyl-5β-oxyethylthiazolemammalian brain.

Заключение

Таким образом, материалы, представленные в этом исследовании, указывают на существование гормон-опосредованной неферментативной функции продукта тиаминазного процесса 4-метил-5β-оксиэтилтиазола мозга млекопитающих.

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