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Thiamine Induces Long-Term Changes in Amino Acid Profiles and Activities of 2-Oxoglutarate and 2-Oxoadipate Dehydrogenases in Rat Brain

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Abstract—Molecular mechanisms of long-term changes in brain metabolism after thiamine administration (single i.p. injection, 400 mg/kg) were investigated. Protocols for discrimination of the activities of the thiamine diphosphate (ThDP)-dependent 2-oxoglutarate and 2-oxoadipate dehydrogenases were developed to characterize specific regulation of the multienzyme complexes of the 2-oxoglutarate (OGDHC) and 2-oxoadipate (OADHC) dehydrogenases by thiamine. The thiamine-induced changes depended on the brain-region-specific expression of the ThDP-dependent dehydrogenases. In the cerebral cortex, the original levels of OGDHC and OADHC were relatively high and not increased by thiamine, whereas in the cerebellum thiamine upregulated the OGDHC and OADHC activities, whose original levels were relatively low. The effects of thiamine on each of the complexes were different and associated with metabolic rearrangements, which included (i) the brain-region-specific alterations of glutamine synthase and/or glutamate dehydrogenase and $NADP^+$-dependent malic enzyme, (ii) the brain-region-specific changes of the amino acid profiles, and (iii) decreased levels of a number of amino acids in blood plasma. Along with the assays of enzymatic activities and average levels of amino acids in the blood and brain, the thiamine-induced metabolic rearrangements were assessed by analysis of correlations between the levels of amino acids. The set and parameters of the correlations were tissue-specific, and their responses to the thiamine treatment provided additional information on metabolic changes, compared to that gained from the average levels of amino acids. Taken together, the data suggest that thiamine decreases catabolism of amino acids by means of a complex and long-term regulation of metabolic flux through the tricarboxylic acid cycle, which includes coupled changes in activities of the ThDP-dependent dehydrogenases of 2-oxoglutarate and 2-oxoadipate and adjacent enzymes.

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The neurotropic effect of thiamine (vitamin B1), which is used in high doses for treatment of neurological disorders including neurodegenerative diseases [1], is mostly considered to be due to the coenzyme action of the diphosphorylated derivative, thiamine diphosphate (ThDP). Studies on the role of ThDP-dependent enzymes in brain pathologies arising due to thiamine deficiency pointed to an essential role of dysfunction of

Abbreviations: GDH, glutamate dehydrogenase; GS, glutamine synthase; MDH, malate dehydrogenase; ME, $NADP^+$-dependent malic enzyme; OADH, 2-oxoadipate dehydrogenase; OADHC, 2-oxoadipate dehydrogenase complex; OGDH, 2-oxoglutarate dehydrogenase; OGDHC, 2-oxoglutarate dehydrogenase complex; PDHC, pyruvate dehydrogenase complex; TCA, tricarboxylic acid; ThDP, thiamine diphosphate.

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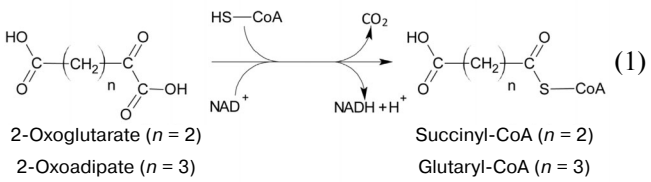
2-oxoglutarate dehydrogenase complex (OGDHC) in the development of such pathologies [2, 3]. Oxidative decarboxylation of 2-oxoglutarate, catalyzed by OGDHC, is important for degradation of amino acids, including those with neurotransmitter functions [4]. In particular, compared to other tissues, the thiamine-deficiency-induced decrease in OGDHC activity in the central nervous system is associated with dysregulated metabolism of the major excitatory neurotransmitter glutamate [5-7] and its neurotoxicity [8]. In addition, perturbed metabolism may stimulate side reactions catalyzed by OGDHC, thus enhancing production of reactive oxygen species [9].

On the other hand, thiamine deficiency causes inflammatory processes in the brain, including microglia activation [10], increase in the expression of CD40/CD40L, proinflammatory cytokines IL-1, TNF, IL-6, and products of arachidonic acid metabolism [11]. These observations may be linked to the recently characterized role of the 2-oxoglutarate dehydrogenase (OGDH) and dihydrolipoamide succinyltransferase components of OGDHC in immune response [12]. In this regard, a new ThDP-dependent dehydrogenase (encoded by the *dhtkd1* gene, homologous to OGDH [13]) deserves attention: it is supposed to function as a component of the 2-oxoadipate dehydrogenase complex (OADHC), which, like OGDHC, would include dihydrolipoamide succinyltransferase and dihydrolipoamide dehydrogenase [14-18]. Accordingly, OADHC is presumed to catalyze a ThDP-dependent reaction similar to that catalyzed by OGDHC (reaction (1)) yet more specific to 2-oxoadipate, a common intermediate of the lysine and tryptophan degradation pathways.



(1)

Accumulation of either 2-oxoglutarate upon mutation-induced deficiencies in OGDHC [19] or 2-oxoadipate upon mutations in *dhtkd1* [14-16] indicates that under physiological conditions OGDHC and OADHC oxidize 2-oxoglutarate and 2-oxoadipate, respectively. However, a high similarity of the active centers and catalytic mechanisms of these homologous enzymes [13] is consistent with oxidation of both 2-oxoglutarate and 2-oxoadipate by classic OGDH encoded by the *ogdh* gene, as has been observed *in vitro*. The similarity also suggests analogous ability of the 2-oxoadipate dehydrogenase (OADH) encoded by the *dhtkd1* gene. The question on the physiologically relevant ratio of the 2-oxoglutarate and 2-oxoadipate dehydrogenase activities of either OGDH or OADH in animal tissues remains unresolved. For the electrophoretically homogeneous multienzyme complex of OGDH from an organism that lacks the *dhtkd1* gene (*Azotobacter vinelandii*), the catalytic rate with 2-oxoadipate was no more than 3% of that with 2-oxoglutarate [20]. In contrast, for OGDH and OGDHC preparations from animal tissues the ratio is increased up to 30% [21, 22]. Because selectivity to 2-oxo acids of 2-oxo acid dehydrogenase complexes from animals is usually significantly higher compared to the bacterial counterparts, the relatively rapid transformation of 2-oxoadipate by OGDH and OGDHC from animal tissues may be due to the presence of a previously unknown OADH encoded by the *dhtkd1* gene in the partially purified OGDHC preparations. OADHC could be therefore involved in a number of functions and pathologies that are commonly attributed to OGDHC. In particular, study of molecular mechanisms of thiamine action requires a differentiation of the OADHC and OGDHC activities in homogenates of analyzed tissues.

This work focuses on the role of the ThDP-dependent dehydrogenases of dicarboxylic 2-oxo acids in long-term regulation of brain metabolism by thiamine. To identify specific contributions of the dehydrogenases of 2-oxoglutarate or 2-oxoadipate to the thiamine-induced metabolic changes in the brain, we developed a method of differential quantification of the OADHC and OGDHC activities in brain homogenates. Selection of the amino acids and several enzymes of central metabolism as biochemical markers of the processes linked to the changed function of the ThDP-dependent dehydrogenases is based on our previous results. We have taken into account data on metabolic regulation by thiamine and its derivatives *in vitro* [23] and *in situ* [24], the role of OGDHC in the metabolism of amino acids [6, 25, 26], and participation of OADHC in catabolism of lysine and tryptophan [18].

MATERIALS AND METHODS

Animals. Our studies were performed on female Sprague–Dawley rats weighing 250-300 g. The animals were kept in standard conditions with 12 h light and 12 h dark cycle in cages with free access to water and meal. Manipulations with rats were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986 ETS No. 123, Strasbourg, 18 March 1986).

Administration of thiamine. Thiamine was injected once intraperitoneally (i.p.) at a dose of 400 mg/kg. For investigation of stable metabolic changes initiated by the high dose of thiamine, the animals were sacrificed eight weeks after the treatment.

Preparation of rat blood plasma. Blood sampling was performed upon decapitation of the rats. Heparin was added to the blood as anticoagulant (10 μl/ml). After cen-

trifugation for 20 min at 4°C and 3220*g*, supernatants of the blood plasma were stored in liquid nitrogen.

Homogenization and extraction of rat brain tissue. After the rats were sacrificed by decapitation, the brain was taken out, put on ice, and rapidly separated into the cerebral hemispheres (further called the cortex), cerebellum, and brain stem. The tissue samples were frozen in liquid nitrogen and stored at −70°C for at least 2 weeks before the enzymatic assays. To analyze the enzymatic activities, the samples were homogenized according to a previously published protocol [5]. Both non-sonicated and sonicated homogenates were analyzed in this work. Under the "sonicated homogenates", we will further refer to samples where mitochondrial proteins were solubilized by sonication and extraction with detergents as described earlier [5]. To analyze the amino acid profiles, the brain cortex and cerebellum of experimental animals were extracted with methanol and acetic acid according to a published procedure [25].

Enzymatic assays. Measurements were performed in 0.2 ml of reaction medium on a Tecan Sunrise microplate reader (Austria). The $NAD(P)^+$-dependent enzymes were assayed under previously described conditions [24] with minor modifications, including substitution of the buffer with 0.1 M Tris-HCl, pH 7.5, for the malate dehydrogenase (MDH) reaction, and with 0.05 M MOPS, pH 7.0, for the OGDHC- and OADHC-catalyzed reactions. The reactions were followed by changes in absorbance of NAD(P)H at 340 nm, using the initial linear parts of the product accumulation curves. For determination of the OADHC activity, 2-oxoglutarate was replaced with 2-oxoadipate. Determination of enzymatic activities included at least three technical replicates for each tissue sample of individual animals. Activities of enzymes are expressed in μmol of the product generated per minute per gram of fresh weight of the tissue. The glutamine synthase (GS) activity was assayed according to [27, 28] by absorbance at 540 nm of the reaction product γ-glutamyl hydroxamate in complex with Fe^{3+} under acidic conditions.

Quantification of amino acids in rat blood plasma and brain extracts. Before chromatography, the extracts and plasma samples were filtered through Vivaspin 500, MWCO 3000 (Sartorius, Germany). The samples were assayed on an L-8800 amino acid analyzer (Hitachi Ltd., Japan) according to the manufacturer's instructions. For HPLC, the 2622SC-PF ion-exchange column (Hitachi Ltd., P/N 855-4507, 4.6 × 60 mm) was eluted at a rate of 0.35 ml/min by step gradients of Li-citrate buffers and temperature (in the range 30-70°C). Multichrom 1.71a software (Ampersand Ltd., Russia) was used to quantify the peaks obtained after chromatographic separation of the extracts.

Statistical analysis. Data on the enzymatic activities and content of amino acids are presented as means ± SEM. Statistical significance of differences between the experimental groups was estimated using the nonparametric Mann–Whitney *U*-test. Correlation analysis was carried out using Statistica 10.0 (StatSoft Inc., USA) with Pearson's test. Statistical significance of differences in the parameters characterizing metabolic interactions between amino acids was assessed by the Wilcoxon signed rank test for paired samples based on continuity correction using RStudio software (RStudio Inc., USA). Differences with $p \leq 0.05$ were considered to be statistically significant.

RESULTS

Differential determination of OADHC and OGDHC activities in rat brain homogenates. As described in the introduction, one of the problems in biochemical investigation of dehydrogenases of dicarboxylic 2-oxo acids is the capability of these enzymes to catalyze reactions with both 2-oxoglutarate and 2-oxoadipate. Hence, in unpurified preparations, one needs to discriminate the activities of the enzymes that are involved in physiological oxidation of 2-oxoglutarate (OGDH encoded by the *ogdh* and *ogdhl* genes) from that of the 2-oxoadipate-specific dehydrogenase (OADH encoded by the *dhtkd1* gene). In particular, this problem arises when the activities are used as biochemical markers of (patho)physiological conditions in tissue homogenates. Searching for the conditions to discriminate the OGDHC and OADHC activities in the brain, we have taken into account the possibility of both the intra- and extramitochondrial localization of the *dhtkd1* gene product that is predicted from the primary structure analysis of the *dhtkd1* protein [13]. Therefore, we investigated the rates of reactions catalyzed by OGDHC and OADHC of the rat brain homogenates both before and after the enzyme solubilization by sonication and detergents. As previously shown [29], the solubilization of intramitochondrial OGDHC in rat brain requires sonication and detergent extraction even after the tissue has been frozen and thawed, which is confirmed by the results presented in Table 1. In most cases when OGDHC is fully solubilized, the OADHC/OGDHC activity ratio decreases 3-4-fold compared to the ratio in non-sonicated homogenates (Fig. 1). Such a drop in the OADHC/OGDHC activity ratio is primarily determined by an increase in the OGDHC activity due to its optimal solubilization after sonication and detergent extraction. However, in most cases, the OADHC activity is notably decreased after this procedure (Table 1). Hence, OADHC is mostly inactivated under conditions of optimal solubilization of OGDHC. As a result, higher OADHC/OGDHC ratios, which are indicative of increased contributions of OADHC to the measured 2-oxoadipate dehydrogenase reaction rate, are observed in the non-sonicated brain homogenates (Fig. 1). In contrast, the maximal OGDHC activity is observed only in the sonicated brain

Table 1. Effect of solubilization conditions on oxidative decarboxylation of 2-oxoadipate (OADHC activity) and 2-oxoglutarate (OGDHC activity) catalyzed by enzymes in homogenates of cerebral cortex, cerebellum, and brain stem of rats

Sonication and detergents	Brain region	Intact rats (n = 7)		Rats after thiamine injection (n = 6)	
		OADHC	OGDHC	OADHC	OGDHC
No	cortex	**0.21 ± 0.05**	0.24 ± 0.04	**0.10 ± 0.03**	0.14 ± 0.03
	cerebellum	**0.05 ± 0.04**	0.21 ± 0.04	**0.22 ± 0.03**	0.45 ± 0.04
	brain stem	**0.08 ± 0.03**	0.18 ± 0.03	**0.12 ± 0.03**	0.22 ± 0.03
Yes	cortex	0.28 ± 0.07	**0.44 ± 0.09**	0.09 ± 0.02	**0.47 ± 0.03**
	cerebellum	0.00 ± 0.02	**0.24 ± 0.05**	0.01 ± 0.02	**0.44 ± 0.02**
	brain stem	0.00 ± 0.04	**0.19 ± 0.02**	0.05 ± 0.04	**0.31 ± 0.06**

Note: The enzymatic activities are given in μmol/min per g fresh weight. Activities determined under conditions of the maximal contribution of each of the complexes to the reaction rate are marked in bold. n, number of animals in groups.

homogenates (Table 1). This difference in optimal solubilization conditions of OGDHC and OADHC enables a relatively specific characterization of the activities of these enzymes in brain homogenates.

Figure 2 compares the activities of OADHC and OGDHC determined in homogenates of different brain regions across the studied experimental groups. Each reaction was measured under its optimal conditions, i.e. before and after the sonication and incubation with detergents for OADHC and OGDHC, respectively. The data indicate that in intact Sprague–Dawley female rats, both the OGDHC and OADHC activities are higher in the cortex than in the cerebellum and brain stem, with no significant difference between the latter two regions (Fig. 2).

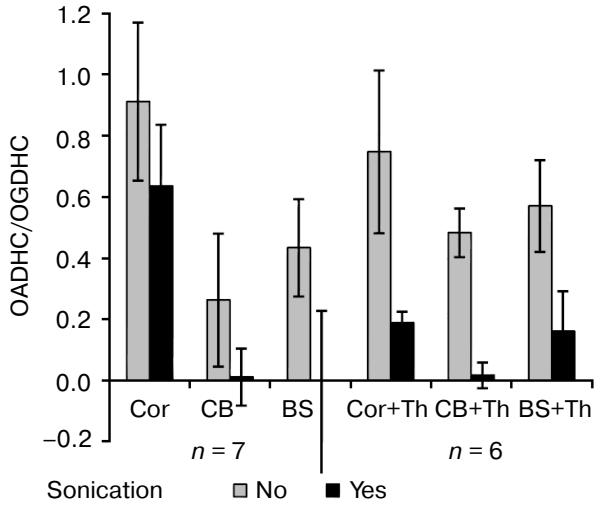


Fig. 1. Ratio of 2-oxoadipate and 2-oxoglutarate oxidative decarboxylation catalyzed by OADHC and OGDHC of homogenates of rat cerebral cortex (Cor), cerebellum (CB), and brain stem (BS) before and after sonication and detergent extraction. +Th, homogenates from rats eight weeks after thiamine injection; n, number of animals in groups.

Changes in OADHC and OGDHC activities in rat brain after thiamine injection. In this study, long-term (eight weeks) effects of a single intraperitoneal injection of a high dose of thiamine (400 mg/kg) to rats were investigated. As seen from Fig. 2, the injection changes the OGDHC and/or OADHC activities in the brain. The difference observed in the regulation of each of these activities (Fig. 2) is evidence of specific assay of OADHC and OGDHC by our protocol. The discrimination of the activities is due to maximization of the contribution of each of these dehydrogenases to the rate of oxidation of their respective substrates under elaborated conditions. According to Fig. 2, in the cortex the thiamine injection decreases the OADHC activity without changing the OGDHC activity. In contrast, in the cerebellum, the thiamine injection increases both activities, but the degree of OADHC activation is higher than that of OGDHC. In the brain stem, only OGDHC activity is significantly increased by thiamine. Thus, the developed protocol of *in vitro* assays of OADHC and OGDHC of the brain homogenates allows us to differentiate the influence of thiamine on these complexes *in vivo*, revealing the independent and brain-region-specific regulation of OADHC and OGDHC in rats after the thiamine injection.

In accordance with previous studies on regulation of 2-oxo acid dehydrogenase complexes *in vivo* [4], the decrease in the OADHC activity in the cortex that we observe eight weeks after a single thiamine injection (Fig. 2) may compensate for a hyper-activation of OADHC due to increased level of the coenzyme ThDP after the injection of its precursor thiamine. The long-term rearrangement of central metabolism may compensate for potential increase in the metabolic flux through ThDP-dependent dehydrogenases in response to thiamine injection into the animals. This is confirmed by changes in activities of several enzymes of central metabolism that are functionally related to ThDP-dependent dehydrogenases and intermediates of the tricarboxylic

acid (TCA) cycle (Fig. 3). Competing for glutamate with glutamine synthase (GS), glutamate dehydrogenase (GDH) produces 2-oxoglutarate, which is oxidized by OGDHC in the TCA cycle. $NADP^+$-dependent malic enzyme (ME) produces pyruvate, which is a substrate of ThDP-dependent pyruvate dehydrogenase complex (PDHC) supplying acetyl-CoA into the TCA cycle. Malate dehydrogenase (MDH, measured as total activity of the cytoplasmic MDH1 and mitochondrial MDH2 isoenzymes) produces oxaloacetate, which incorporates acetyl-CoA to form citric acid (Fig. 3e). Figure 3 shows that in the cerebral cortex the activities of GS and GDH are reduced after the thiamine injection, whereas the ME activity is increased. Other changes in the activities are observed in the cerebellum, where the thiamine injection decreases the activities of ME and GDH without affecting MDH and GS (Fig. 3, a-d). Thus, not only OADHC and OGDHC, but also a number of functionally related enzymes (Fig. 3e) demonstrate long-term changes in animals after a single thiamine injection, and these changes are different in the cortex and cerebellum.

Amino acids in the brain as metabolic markers of long-term effect of thiamine administration. For several reasons, free amino acids are sensitive biochemical markers of changes in activities of ThDP-dependent dehydrogenases of 2-oxo acids in rat brain. First, degradation of amino acids occurs in the TCA cycle, where OGDHC limits the metabolic flux [8]. Second, the catabolism of many amino acids initiated by or occurring through transamination into pyruvate, 2-oxoglutarate, 2-oxoadipate, and branched-chain 2-oxo acids is strongly coupled to irreversible action of the 2-oxo acid dehydrogenases [4]. Indeed, the brain-region-specific differences in the thiamine effects on OADHC, OGDHC, and functionally associated enzymes (Figs. 2 and 3) are accompanied by changes in amino acid profiles, which are also region-specific. In rat cortex, levels of many amino acids are decreased after the thiamine administration, and some amino acids demonstrated a similar decrease in the cerebellum (Table 2). However, alanine content is elevated in the cortex ($p < 0.05$) as well as in the cerebellum ($p = 0.07$) in response to thiamine. Increase in alanine often indicates a reduction in the metabolic flux through the TCA cycle [26]. Nevertheless, in the cortex accumulation of alanine may be also due to the changed function of affiliated enzymes, such as the increased activity of the pyruvate-generating ME, and the reduced activities of both GDH and GS, potentially affecting the production of 2-oxoglutarate from glutamate (Fig. 3). As noted above, GS has a common substrate with the GDH reaction and utilizes ammonium generated in the catabolism of nitrogen compounds in the brain [30]. Together with the increased level of alanine, the decrease in both amino acids content and GS activity in the cortex suggest a reduction in the amino acid catabolism in this brain region after the thiamine injection. This conclusion is consistent with a long-term decrease in the activity of OADHC, which takes part in the catabolism of lysine and tryptophan [16, 22] (Fig. 2). The changes in the enzymatic activities in the cerebellum (Figs. 2 and 3) point to another metabolic rearrangement in this brain region, compared to cortex, which is also confirmed by the quantification of the cerebellar amino acids (Table 2). Indeed, the GDH and ME activities in the cerebellum are reduced by thiamine, whereas the GS activity does not change (Fig. 3). Thiamine also increases the OADHC and OGDHC activities of the cerebellum (Fig. 2). Although OADHC and OGDHC participate in degradation of lysine, tryptophan, and glutamate, with the latter being a specific metabolic indicator of alterations in the OGDHC activity [8, 31], the levels of these amino acids remain constant (Table 2). However, it should be noted that even after the increases in the OGDHC and OADHC activities caused by thiamine in the cerebellum and brain stem, the levels of these activities are not higher than in the cortex of intact rats (Fig. 2). Apparently, this level determines a maximal limit for the physiological variations in the OADHC and OGDHC activities that occur in the brain. Up to this limit, changes in the OADHC and OGDHC activities (Fig. 2) may be appropriately compensated by regulation of activities of other enzymes (Fig. 3). Such compensation is obviously required for brain homeostasis, as it stabilizes levels of essential metabolites, including a wide range of amino acids in the cerebellum (Table 2).

Thus, the thiamine injection to animals results in different alterations of the activities of the 2-oxoglutarate and 2-oxoadipate dehydrogenases (Fig. 2). Tissue-specif-

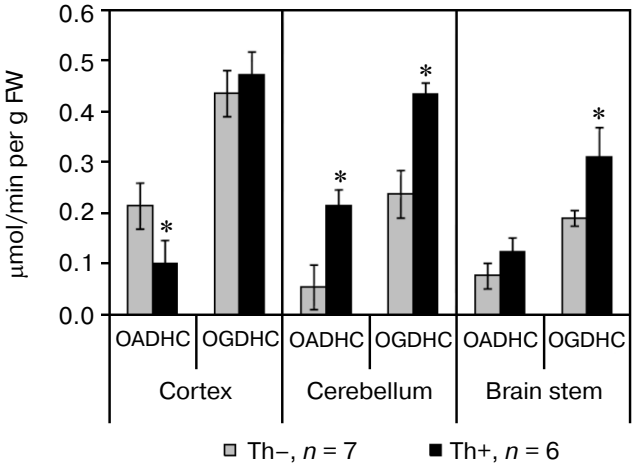


Fig. 2. Rates of 2-oxoadipate and 2-oxoglutarate oxidative decarboxylation catalyzed by OADHC and OGDHC in homogenates of rat brain cortex, cerebellum, and brain stem measured under conditions optimized for each of the activities. Th–, homogenates from intact rats; Th+, homogenates from rats eight weeks after thiamine injection; *n*, number of animals in groups; *, significant ($p \leq 0.05$) differences between intact and thiamine-treated rats.

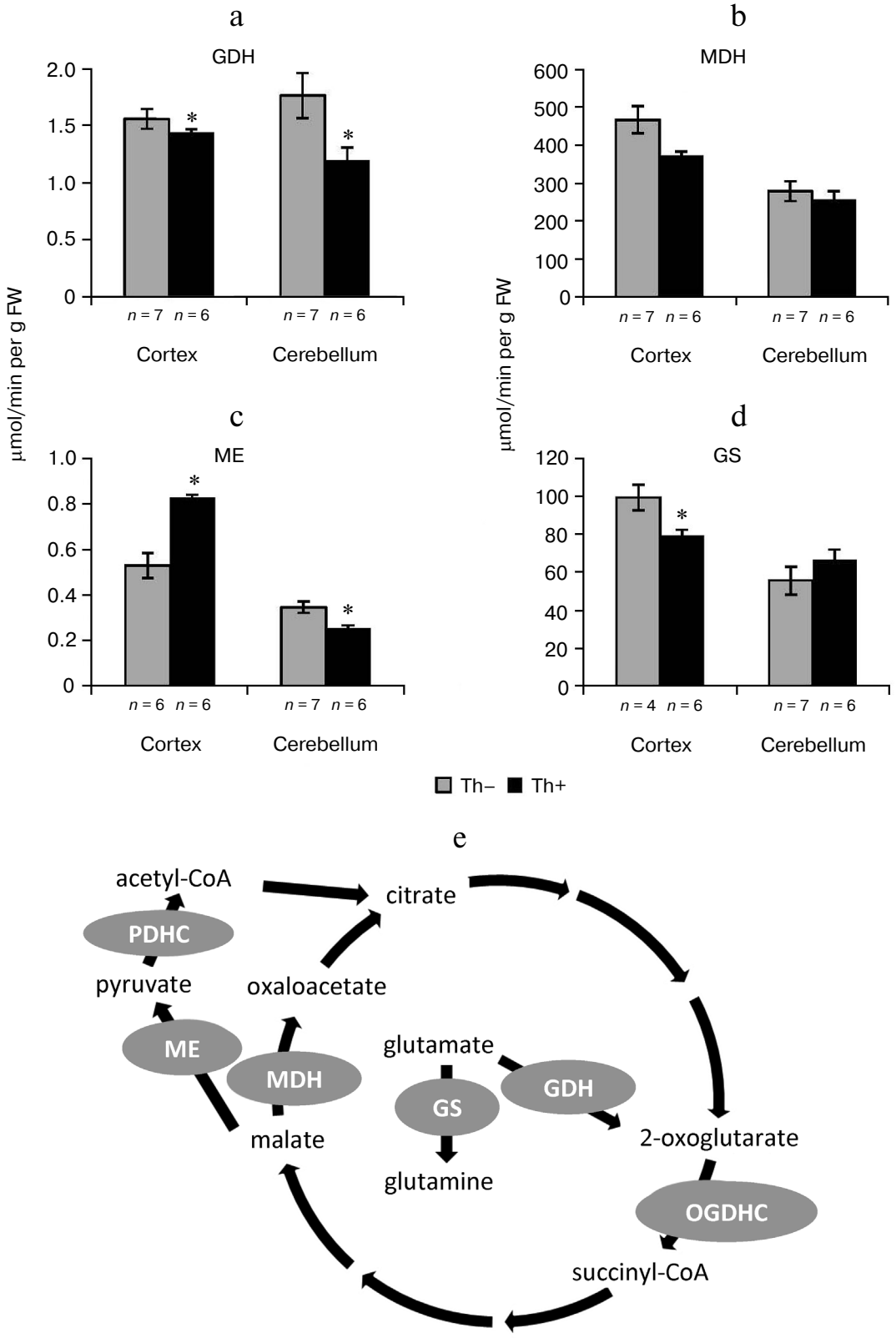


Fig. 3. Thiamine-induced changes in activities of central metabolic enzymes assayed in homogenates of rat cerebral cortex and cerebellum: a) glutamate dehydrogenase (GDH); b) malate dehydrogenase (MDH); c) $NADP^+$-dependent malic enzyme (ME); d) glutamine synthase (GS); e) role of the enzymes in the TCA cycle which substrate acetyl-CoA is produced by ThDP-dependent pyruvate dehydrogenase complex (PDHC). Th–, homogenates from intact rats; Th+, homogenates from rats eight weeks after thiamine injection; *n*, number of animals in groups; *, significant ($p \leq 0.05$) differences between intact and thiamine-treated rats.

Table 2. Changes in amino acid profiles of blood plasma, brain cortex, and cerebellum of Sprague–Dawley rats eight weeks after thiamine injection

Amino acids	Blood plasma			Cortex			Cerebellum		
	control $n = 7$	thiamine $n = 6$	p	control $n = 7$	thiamine $n = 6$	p	control $n = 7$	thiamine $n = 6$	p
Ala	359 ± 13	329 ± 34	0.534	0.57 ± 0.05	0.78 ± 0.05	***0.014*** ↑	0.57 ± 0.05	0.69 ± 0.06	*0.073* ↑
Arg	188 ± 8	177 ± 9	0.534	0.17 ± 0.01	0.11 ± 0.004	***0.002*** ↓	0.17 ± 0.02	0.13 ± 0.01	*0.101* ↓
Asp	24 ± 3	16 ± 3	*0.101* ↓	2.00 ± 0.11	2.09 ± 0.17	0.731	1.64 ± 0.15	1.61 ± 0.08	0.731
Cystine	n.d.	n.d.	–	0.017 ± 0.002	0.008 ± 0.001	***0.008*** ↓	0.03 ± 0.002	0.013 ± 0.001	***0.001*** ↓
GABA	n.d.	n.d.	–	3.11 ± 0.32	2.21 ± 0.19	***0.035*** ↓	2.43 ± 0.29	2.09 ± 0.11	0.836
Gln	562 ± 15	599 ± 20	0.138	3.75 ± 0.30	4.14 ± 0.21	0.445	3.55 ± 0.23	4.04 ± 0.14	*0.101* ↑
Glu	98 ± 5	70 ± 9	***0.022*** ↓	8.82 ± 0.63	9.34 ± 0.45	0.836	7.30 ± 0.50	8.02 ± 0.29	0.366
Gly	316 ± 15	200 ± 12	***0.001*** ↓	0.94 ± 0.08	2.20 ± 1.47	0.366	0.97 ± 0.15	0.74 ± 0.05	0.295
His	68 ± 2	62 ± 4	*0.073* ↓	0.07 ± 0.01	0.06 ± 0.003	0.534	0.07 ± 0.01	0.07 ± 0.01	0.628
Ile	79 ± 3	63 ± 4	***0.022*** ↓	0.05 ± 0.004	0.04 ± 0.003	***0.014*** ↓	0.05 ± 0.01	0.04 ± 0.01	0.295
Leu	126 ± 5	100 ± 6	***0.005*** ↓	0.10 ± 0.01	0.07 ± 0.003	***0.005*** ↓	0.11 ± 0.02	0.07 ± 0.01	*0.101* ↓
Lys	484 ± 16	400 ± 21	***0.014*** ↓	0.24 ± 0.02	0.16 ± 0.01	***0.014*** ↓	0.32 ± 0.03	0.31 ± 0.02	0.836
Met	55 ± 4	49 ± 8	0.295	0.06 ± 0.01	0.04 ± 0.007	***0.008*** ↓	0.06 ± 0.01	0.04 ± 0.002	*0.073* ↓
Phe	62 ± 1	52 ± 3	***0.014*** ↓	0.06 ± 0.01	0.04 ± 0.006	***0.001*** ↓	0.07 ± 0.01	0.06 ± 0.004	0.234
Ser	293 ± 9	242 ± 13	***0.022*** ↓	0.79 ± 0.08	0.97 ± 0.06	0.234	0.60 ± 0.08	0.69 ± 0.07	0.138
Thr	292 ± 6	174 ± 18	***0.001*** ↓	0.59 ± 0.05	0.44 ± 0.02	***0.035*** ↓	0.63 ± 0.04	0.46 ± 0.03	***0.014*** ↓
Trp	74 ± 1	47 ± 2	***0.001*** ↓	0.014 ± 0.001	0.008 ± 0.001	***0.001*** ↓	0.014 ± 0.002	0.017 ± 0.002	0.138
Tyr	49 ± 4	37 ± 2	***0.022*** ↓	0.05 ± 0.01	0.03 ± 0.002	***0.001*** ↓	0.05 ± 0.01	0.05 ± 0.01	0.628
Val	178 ± 6	132 ± 10	***0.008*** ↓	0.10 ± 0.01	0.09 ± 0.01	0.366	0.11 ± 0.02	0.09 ± 0.01	0.295

Note: Amino acids are given in alphabetical order. Amino acid quantities in extracts of cortex and cerebellum are presented as μmol per g fresh weight, in plasma – as μmol per liter. n, number of animals in groups; n.d., not determined. Quantities of cystine and γ-aminobutyric acid (GABA) in blood plasma were too low to detect. The direction of changes in the thiamine-treated group of rats compared to control is indicated by the upwards or downwards arrows next to the p-values for $p \le 0.1$. Significant differences ($p \le 0.05$) and trends ($p \le 0.1$) are indicated in bold italics and italics, respectively.

ic homeostasis is maintained by the regulation of ThDP-dependent dehydrogenases of 2-oxo acids and associated enzymes (Fig. 3). The changes in the activities of these enzymes are directed to maintain the amino acid levels in the cerebellum (Table 2) where thiamine activated the originally low levels of the OADHC and OGDHC activities. However, in the cortex, where the activities are initially high, a long-term compensatory regulation in response to thiamine also involves decreases in the content of a number of amino acids (Table 2). A demand for a more profound compensation of the activating action of thiamine on the dehydrogenases of 2-oxo acids in the cortex is met by a long-term reduction of the activity of OADHC and no increase in the activity of OGDHC (Fig. 2).

Amino acids of blood plasma as indicators of metabolic regulation of the whole organism by thiamine. Unlike the cortex or cerebellum, where the content of metabolic intermediates could depend on tissue-specific metabolism (Table 2), the metabolome of the blood plasma reflects the general state of an organism. Long-term changes in the amino acid profile of the blood plasma caused by the thiamine injection show a high degree of similarity with the changes observed in the cortex. In both cases, levels of the amino acids whose degradation is directly linked to ThDP-dependent dehydrogenases of 2-oxo acids, are decreased. Those include amino acids of the pyruvate group, branched-chain amino acids, lysine, and tryptophan. For some of these amino acids, a trend ($p \le 0.1$) to decreased levels could also be seen in the cerebellum (Table 2), but the changes do not reach statistical significance ($p \le 0.05$). Remarkably, the glutamate content decreases in the blood plasma, but not in the cortex or the cerebellum. Obviously, constant level of glutamate in the brain is required due to its neurotransmitter function, whereas in the plasma the glutamate level is determined also by metabolism of other tissues. On the other hand, the alanine level is increased in the brain, but it is not changed in blood plasma (Table 2). The elevation of the alanine content that is observed in the brain is there-

Table 3. Analysis of changes in correlations between amino acid levels in blood plasma, cerebral cortex, and cerebellum of Sprague–Dawley rats before (Th–, $n = 7$) and eight weeks after (Th+, $n = 6$) thiamine injection

Amino acids	Blood plasma								Cortex								Cerebellum							
	Σ		$\bar{X}$		–		+		Σ		$\bar{X}$		–		+		Σ		$\bar{X}$		–		+	
	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+	Th–	Th+
Ala	7.1	8.8	0.44	0.55	0	0	1	0	13.6	12.7	0.76	0.71	0	0	11	10	13.9	13.4	0.78	0.75	0	0	12	8
Arg	9.3	1.2	0.58	0.07	1	0	5	0	13.4	12.7	0.74	0.70	0	0	10	9	12.7	13.2	0.71	0.73	0	0	11	8
Asp	5.9	5.2	0.37	0.33	1	0	4	1	12.0	14.0	0.67	0.78	0	0	4	12	13.4	8.8	0.74	0.49	0	0	11	0
Cystine	–	–	–	–	–	–	–	–	12.4	12.7	0.69	0.71	0	0	5	8	11.4	7.4	0.63	0.41	0	0	5	0
GABA	–	–	–	–	–	–	–	–	11.7	13.0	0.65	0.72	0	0	5	10	13.6	12.6	0.75	0.70	0	0	12	6
Gln	–8.5	10.5	–0.53	0.65	4	0	0	7	14.0	14.2	0.78	0.79	0	0	11	13	8.0	13.2	0.44	0.74	0	0	5	5
Glu	7.7	7.6	0.48	0.48	0	0	2	1	11.4	14.0	0.64	0.78	0	0	6	12	4.2	13.5	0.23	0.75	0	0	3	6
Gly	5.8	4.1	0.36	0.26	0	0	3	0	12.1	5.9	0.67	0.33	0	0	8	0	6.8	14.9	0.38	0.83	0	0	0	13
His	4.2	11.3	0.27	0.71	1	0	2	8	14.5	10.0	0.81	0.56	0	0	11	2	12.7	13.4	0.70	0.74	0	0	11	8
Ile	6.6	11.9	0.41	0.74	0	0	2	9	12.5	13.4	0.70	0.74	0	0	7	11	11.6	13.3	0.65	0.74	0	0	10	9
Leu	6.0	11.0	0.38	0.69	0	0	2	8	14.9	13.1	0.83	0.73	0	0	14	11	12.0	14.5	0.67	0.81	0	0	10	11
Lys	4.8	6.7	0.30	0.42	0	0	2	1	11.7	13.7	0.65	0.76	0	0	8	11	8.1	14.1	0.45	0.78	0	0	5	8
Met	7.7	9.6	0.48	0.60	1	0	6	6	13.5	13.0	0.75	0.72	0	0	11	11	12.2	14.4	0.68	0.80	0	0	10	11
Phe	8.6	11.4	0.54	0.71	0	0	3	6	15.1	11.0	0.84	0.61	0	0	12	3	13.0	13.9	0.72	0.77	0	0	11	10
Ser	9.5	10.4	0.59	0.65	0	0	7	5	13.0	13.4	0.72	0.75	0	0	10	11	13.3	14.8	0.74	0.82	0	0	12	11
Thr	6.6	10.3	0.41	0.64	0	0	0	9	15.1	7.8	0.84	0.43	0	0	16	0	12.8	11.9	0.71	0.66	0	0	7	3
Trp	4.5	10.6	0.28	0.66	0	0	1	6	11.4	3.7	0.63	0.20	0	0	8	0	3.7	12.2	0.20	0.68	0	0	1	8
Tyr	7.7	6.7	0.48	0.42	0	0	3	0	12.9	5.4	0.71	0.30	0	0	9	0	12.3	13.2	0.68	0.73	0	0	10	9
Val	9.4	11.5	0.59	0.72	0	0	5	9	14.8	14.0	0.82	0.78	0	0	14	12	13.3	14.2	0.74	0.79	0	0	12	10
Sum/ average	**102.9**	**148.7**	**0.38**	**0.55**	**8**	**0**	**48**	**76**	**250.0**	**217.4**	**0.76**	**0.71**	**0**	**0**	**180**	**146**	**208.7**	**246.8**	**0.61**	**0.72**	**0**	**0**	**158**	**144**
p	**0.019**		**0.022**		**0.048**		0.154		0.142		0.142		1.000		0.348		**0.028**		**0.030**		1.000		0.274	

Note: Amino acids are given in alphabetical order. For each amino acid, the sum of correlation coefficients (Σ), average correlation coefficient ($\bar{X}$), and total number of statistically significant negative (–) and positive (+) correlations are shown. At the bottom of the table, the sum of Σs, average of $\bar{X}$s, and sum of all positive and negative correlations for all amino acids in each experimental group are listed, and *p*-values of significance of the differences between Th– and Th+ groups are indicated. Data for cystine and γ-aminobutyric acid (GABA) in the blood plasma are not shown as the quantities of these amino acids were too low to detect.

fore not an indicator of a downregulation of the TCA cycle at the whole-organism level.

In general, the changed amino acid profile of the blood that supplies tissues with amino acids suggests that the thiamine administration induces a long-term metabolic reorganization of the whole organism to limit degradation of amino acids through ThDP-dependent dehydrogenases of 2-oxo acids. Nonetheless, decreases in amino acids in the blood plasma are not necessarily accompanied by those in tissues. The specificity of the thiamine-induced metabolic rearrangements in the cortex and cerebellum is obvious from both the amino acid profiles (Table 2) and the profile-linked activities of OGDHC, OADHC, and the enzymes associated with the TCA cycle or its intermediates (Figs. 2 and 3).

Correlation analysis of tissue-specific metabolism of amino acids. Multiple changes in the amino acid profiles of tissues after the thiamine injection depend, in particular, on significant interactions between the pathways of amino acid metabolism, which are, e.g., due to the common transporters or the TCA cycle-mediated degradation of amino acids. In a sample of *n* animals under given experimental conditions, correlated levels of certain amino acids may serve as a measure of such metabolic interactions. Metabolic interactions of each amino acid can be quantified by the sum of its correlation coefficients with other amino acids (Σ), the average correlation coefficient ($\bar{X}$), and the total number of statistically significant negative and positive correlations. Metabolic interactions between all amino acids in a given physiological state can be characterized by the sums or average values of the parameters determined for each amino acid. Quantification of the metabolic interactions between amino acids in the investigated groups of experimental animals is shown in Table 3. According to these data, the thiamine administration changes the correlations

between amino acids in the studied brain regions. In the cortex, where the number of total correlations is originally higher than in the cerebellum, thiamine reduces the number more (by 19%) than it does in the cerebellum (by 9%). However, in both cases these changes in the total numbers of correlations are not statistically significant (Table 3). On the other hand, considering the summarized and average correlation coefficients, one can reveal the statistically significant elevation of metabolic interactions between amino acids in the cerebellum after the thiamine injection. For example, the sum of the correlation coefficients of all amino acids in the cerebellum increases from 209 to 247, and the average correlation coefficient – from 0.61 to 0.72 (Table 3). It should be noted that in the absence of significant changes in overall metabolic interactions between amino acids in the cortex, thiamine dramatically affects the distribution of such correlations among amino acids. The levels of five amino acids – glycine, histidine, threonine, tyrosine and tryptophan – become almost completely independent of the other amino acids after the thiamine injection (Table 3). In the cerebellum, the distribution of statistically significant correlations showed fewer changes after the thiamine injection. Nevertheless, in the cerebellum, a low level of metabolic interactions is shown by glycine and tryptophan, whereas after the thiamine administration the lack of interactions is shifted to aspartate and cystine.

Thus, thiamine induces considerable changes in average levels of amino acids in the cortex (Table 2), where their overall metabolic interactions do not change significantly (Table 3). In contrast, the relative stability of average levels of amino acids in the cerebellum (Table 2) is accompanied by an increase in their metabolic interactions (Table 3).

Figure 4 shows the changes in correlation coefficients characterizing metabolic interaction of glutamate, which is degraded through OGDHC, and lysine and tryptophan, whose degradation pathways include OADHC. According to Table 3, such parameters of metabolic interactions as the total and average correlation coefficients and the number of correlations are increased after the thiamine administration for glutamate and lysine both in the cortex and cerebellum. Nevertheless, increases in both parameters are much higher in the cerebellum (3-fold for glutamate and 2-fold for lysine) than in the cortex (~20% for glutamate and lysine). In the cerebellum, low correlations of glutamate and lysine with other amino acids in intact rats rise dramatically after the thiamine administration, reaching positive values typical for the statistically significant correlations (Fig. 4, b and d). For instance, this is observed for the glutamate correlations with alanine, glycine, histidine, branched-chain amino acids, phenylalanine, methionine, serine, and tyrosine (Fig. 4b). As discussed above, the changes are less manifested in the cortex (Fig. 4, a and c). In the case of tryptophan, thiamine increases the parameters of its metabolic interaction with other amino acids only in the cerebellum, whereas in the cortex the correlations of tryptophan with other amino acids decrease (Fig. 4, e and f). This trend is also revealed by the thiamine-induced changes in the overall correlation parameters of tryptophan shown in Table 3. The quantitative and qualitative differences in the effects of thiamine on the amino acid markers of OGDHC and OADHC function in the cortex and cerebellum agree well with the brain-region-specific influence of thiamine on the activities of OGDHC and OADHC (Fig. 2). In the cerebellum, where the injection of thiamine increases activities of both OGDHC and OADHC (Fig. 2), considerable changes in metabolic interactions between the amino acid markers of OADHC and OGDHC function are observed (Fig. 4). In the cortex, where thiamine does not elevate the OGDHC activity and decreases the OADHC activity (Fig. 2), only metabolic interactions of tryptophan, which is presumed to be a marker of the OADHC function, are found to change dramatically (Fig. 4).

It should be noted that no significant negative correlations between amino acids are detected in the brain of either intact or thiamine-treated rats (Table 3). Nevertheless, the four significant negative correlations and a negative value of the average coefficient of the correlations between glutamine and other amino acids (–0.5) are inherent in the blood plasma of intact rats (Table 3). Because glutamine accepts ammonium arising from degradation of nitrogen compounds [30], the negative correlations mean that in intact rats, glutamine accumulation occurs at the expense of the degradation of amino acids. Remarkably, after the thiamine injection the average correlation coefficient of glutamine becomes positive (0.7), with seven positive correlations being statistically significant (Table 3). Such a change of the glutamine metabolic interaction with other amino acids in the blood favors a decrease in the overall degradation of amino acids in rats after the thiamine administration.

As a result, the correlation analysis reveals metabolic rearrangements caused by the thiamine administration (Table 3), which are consistent with the results of enzymatic assays (Figs. 2 and 3) and are detectable even when the average levels of amino acids do not change significantly (Table 2; cerebellum).

DISCUSSION

OADHC is a new ThDP-dependent multienzyme complex. Structural and functional differences of protein products of the previously characterized *ogdh(l)* and *dhtkd1* genes [13] indicate that the biologically significant oxidation of 2-oxoadipate is catalyzed by the *dhtkd1* gene product [14, 16, 32]. Nevertheless, in many publications on 2-oxoadipate metabolism its oxidation is still attributed to the 2-oxoglutarate dehydrogenase (the isoen-

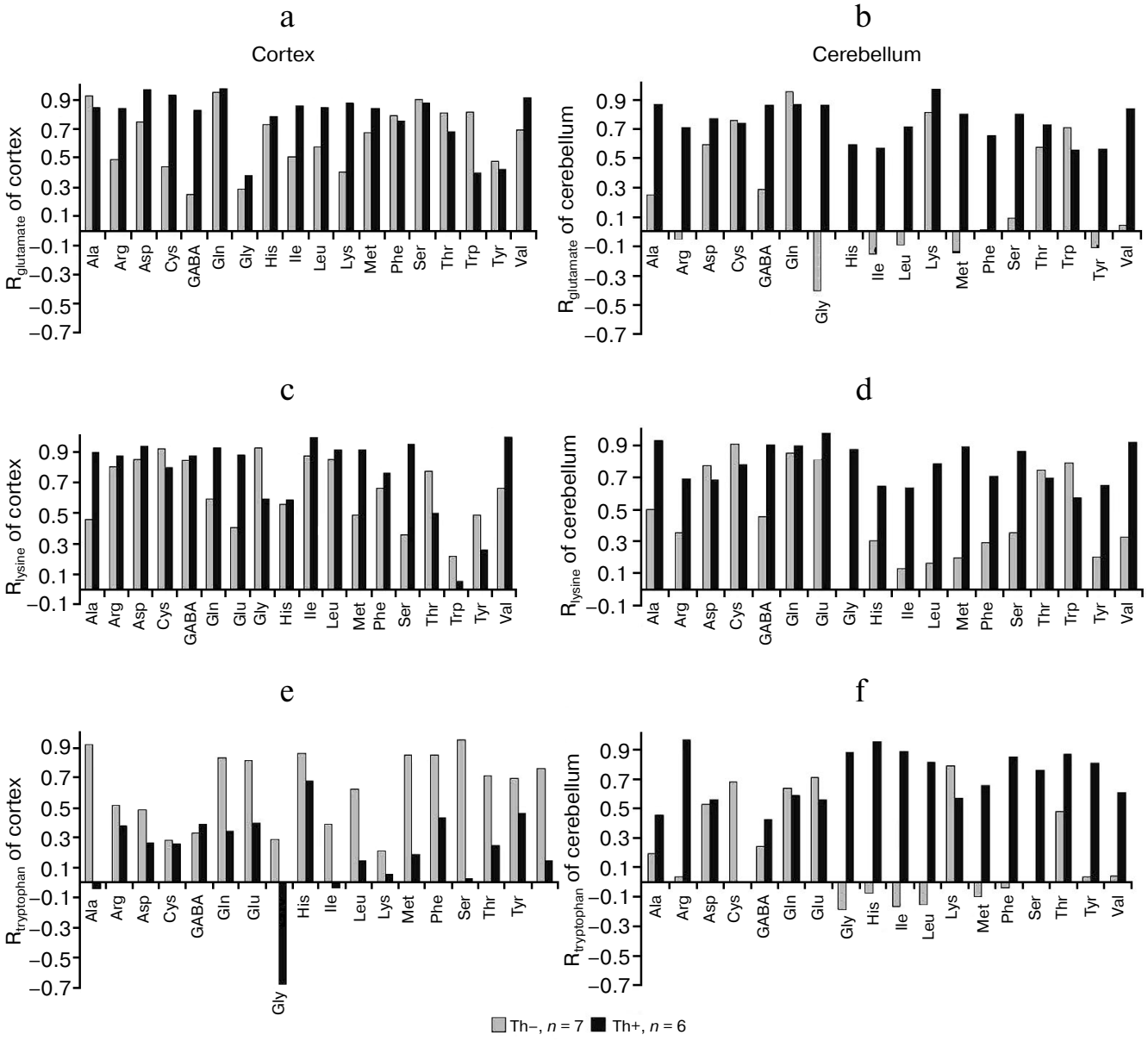


Fig. 4. Effect of thiamine on correlation coefficients (R) of glutamate (a, b), lysine (c, d), and tryptophan (e, f) with other amino acids in the cortex (bar charts on the left) and cerebellum (bar charts on the right). Th–, homogenates from intact rats; Th+, homogenates from rats eight weeks after thiamine injection; *n*, number of animals in groups.

zymes encoded by the *ogdh* and *ogdhl* genes). Moreover, in databases on transcriptomics and other screening studies, the protein encoded by *dhtkd1* is often incorrectly mentioned as 2-oxoglutarate dehydrogenase. Indeed, the biological role of the enzyme encoded by the *dhtkd1* gene is not well understood. Using genetic methods, it was found that deficiency of this enzyme results in disturbances in mitochondrial energy metabolism [32], which in turn lead to neurological symptoms [17, 32]. Mutations of the *dhtkd1* gene cause accumulation of 2-aminoadipate and 2-oxoadipate, which are common intermediates in pathways of the degradation of the essential amino acids lysine and tryptophan [14-16, 18, 33]. Hence, the *dhtkd1* enzyme is supposed to participate in the tissue-specific metabolism of these amino acids. Our results show that OADHC activity, its regulation by thiamine, and the role of OADHC in maintenance of the levels of lysine and tryptophan vary in different regions of the brain (Figs. 2 and 4 and Table 1). This finding suggests that the OADHC-related features of metabolic networks support functional heterogeneity of the brain.

In view of the ability of OGDHC to catalyze the 2-oxoadipate dehydrogenase reaction *in vitro* [21, 22], the main problem in studies on the biological function and

regulation of the *dhtkd1*-encoded OADH is discrimination of the activities inherent in the *dhtkd1*-encoded enzyme, on one hand, and the highly expressed in different tissues OGDH encoded by the *ogdh* and *ogdhl* genes, on the other hand. In this work, we found the conditions for specific assays of the activities of the multienzyme complexes of OADH and OGDH in brain homogenates. Non-sonicated homogenates of the tissues stored at −70°C were shown to be optimal for measuring the OADHC activity. OADHC, which is assayed in such homogenates, is either extramitochondrial, in accordance with the prediction of dual localization of the enzyme [13], or solubilized from mitochondria already after a single cycle of tissue freezing and thawing. The latter condition is insufficient to solubilize intramitochondrial OGDHC. Due to at least partial intramitochondrial localization of OADHC [13, 34], the lack of increase in the OADHC activity in most of the studied samples after sonication and detergent extraction (Table 1) favors the assumption on easier, compared to OGDHC, solubilization of OADHC. In general, low levels of the OADHC activity in the non-sonicated brain homogenates (Table 1) are correlated with a relatively low expression of the *dhtkd1* gene in the brain according to the PaxDB database [35]. Nevertheless, our study has shown that in the brain of Sprague–Dawley female rats the OADHC activity is higher in the cerebral cortex compared to the cerebellum and brain stem (Figs. 1 and 2; intact rats).

Sonication and detergent extraction are necessary to solubilize a major part of OGDHC from brain homogenates. After this treatment, the OADHC activity remains only in the cerebral cortex, whereas OADHC in the cerebellum and brain stem is inactivated (Table 1). The inactivation does not depend on the level of the OADHC activity in the studied brain regions. For example, in thiamine-treated animals, the OADHC activity in the cerebellum and brain stem before sonication is comparable to the OADHC activity in the cerebral cortex of intact rats (Fig. 2). However, both the relatively high levels of OADHC activity in the thiamine-treated rats and the relatively low levels of OADHC activity in intact rats are decreased after the sonication and detergent extraction in the cerebellum and brain stem (Table 1). In contrast, OADHC of the cortex seems to be resistant to the sonication and detergent extraction (Table 1). This may be due to its higher, compared to the enzyme in the cerebellum and brain stem, endogenous saturation by the coenzyme ThDP, which is a known stabilizer of ThDP-dependent dehydrogenases. This suggestion is supported by a lower exchange rate of the thiamine pool (and ThDP as its major representative) in the cortex compared to the cerebellum and brain stem [36].

The finding that after sonication and detergent extraction, the cerebellum and brain stem of intact rats had high (0.19 and 0.24 μmol/min per g fresh weight, respectively) OGDHC activity, but no OADHC activity (Table 1), shows that OGDHC in the rat brain homogenates does not have a detectable level of 2-oxoadipate dehydrogenase activity. A further argument supporting this conclusion comes from comparison of the OADHC activity in sonicated homogenates of the cortex of intact rats and cerebellum of thiamine-treated rats. Having the same level of the OGDHC activity (0.44 μmol/min per g fresh weight), the samples significantly differ in the OADHC activity (0.28 and 0.01 μmol/min per g fresh weight, respectively) (Table 1). Obviously, such variations in the OADHC activity at a constant level of the OGDHC activity point to the involvement of the different catalytic systems, OGDHC and OADHC, into oxidation of 2-oxoglutarate and 2-oxoadipate. Because OGDHC of the brain strongly interacts with the inner membrane of mitochondria [37], which requires sonication and detergent extraction to solubilize OGDHC, the oxidative decarboxylation of both 2-oxoadipate and 2-oxoglutarate in non-sonicated homogenates of the brain samples appears to be catalyzed by the *dhtkd1*-encoded enzyme. In this case, the average ratio of the reaction rates with 2-oxoadipate and 2-oxoglutarate for OADH is about 0.5 (Fig. 1). On the other hand, the variations in the ratios of the OADHC and OGDHC activities observed in non-sonicated homogenates of different experimental groups (Fig. 1) could be due to a varied minor part of OGDHC that has been solubilized in the non-sonicated homogenates. However, as shown above, even a relatively high level of the OGDHC activity (0.44 μmol/min per g fresh weight) does not provide a measurable oxidation of 2-oxoadipate (Table 1; cerebellum of thiamine-treated rats). Hence, the OADHC activity in the non-sonicated homogenates is due to the catalytic action of the enzyme encoded by the *dhtkd1* gene, regardless of the possibility of a low-level partial solubilization of OGDHC under the assay conditions. In this case, only the question whether the *dhtkd1*-encoded OADH transforms 2-oxoglutarate remains open.

Recent studies have shown that the *dhtkd1* gene and/or *dhtkd1*-encoded enzyme are involved in the development of diabetes and treatment of obesity [33, 38]. Because thiamine is also involved in these processes [39], the enzymatic assays elaborated in this work represent new tools to study molecular mechanisms underlying potential role of ThDP-dependent OADH in diabetes and obesity. As a result, the new ThDP-dependent dehydrogenase encoded by the *dhtkd1* gene may be an interesting target for metabolic regulation.

Thiamine as a metabolic regulator. ThDP is a coenzyme of oxidative decarboxylation of 2-oxo acids, including 2-oxoadipate and 2-oxoglutarate. Accordingly, its precursor thiamine (vitamin B1) is a physiological activator of ThDP-dependent 2-oxo acid dehydrogenases. In several previous studies about regulation of multienzyme complexes of 2-oxo acid dehydrogenases, we have shown that the exposure of animals to effectors of these key

metabolic enzymes evokes homeostatic mechanisms [4]. Such mechanisms compensate for the effector-induced perturbations in the oxidative decarboxylation of 2-oxo acids, normalizing changed fluxes by switching on natural mechanisms of attenuation [5, 28]. Differences in the metabolic networks of the cortex, cerebellum, or brain stem may cause different perturbations and compensatory responses to a physiological activator of 2-oxo acid dehydrogenases, thiamine. The differences are primarily seen in the OADHC and OGDHC activities of the corresponding homogenates measured *in vitro* (Fig. 2).

However, the involvement of thiamine in regulation of cell metabolism is not limited to its widely known role as a precursor of the coenzyme for several enzymes of central metabolism. The energy-dependent biosynthesis of non-coenzyme derivatives of thiamine, such as its triphosphorylated and adenylated forms [40, 41], supports additional functions of this vitamin in living systems. It was shown *in vitro* [23] and *in situ* [24] that thiamine itself and/or its derivatives regulate a number of central metabolic enzymes that do not require ThDP as their coenzyme. Results presented in Fig. 3 demonstrate that thiamine injection to rats also induces a long-term *in vivo* regulation of these enzymes.

Recent studies have shown regulatory interactions between the ThDP-dependent OGDHC and thiamine metabolism [31]. In particular, inhibition of OGDHC is compensated by increased uptake of thiamine, both in cultured astrocytes and in rat brain. Thus, short-term changes in activities of ThDP-dependent enzymes may launch long-term processes compensating for the changes. In the present work, we have characterized such long-term processes initiated by a high dose of thiamine. Systemic long-term effects of thiamine may involve a number of mechanisms in addition to those dependent on the coenzyme type of ThDP binding. According to a recent study, the most representative common feature of rat brain proteins that bind to a thiamine-modified sorbent is "acetylation" [23]. Involvement of thiamine in metabolic regulation by this posttranslational modification may underlie the long-term effects of thiamine observed in this work (Figs. 2 and 3). Besides, ThDP is known to bind to a master regulator of metabolism, transcription factor p53, competing with the p53 binding to DNA [42]. On the other hand, thiamine metabolism interacts with mTOR signaling, which senses cellular availability of amino acids [43]. Thus, the long-term effects of a single high dose of thiamine on central metabolic enzymes and amino acid homeostasis in the rat brain, which have been established in our work, may manifest metabolic reprogramming controlled by acetylation, p53, and mTOR.

Correlation analysis of amino acid levels to characterize metabolic changes. Multiple interactions between the pathways of amino acid metabolism include competition of structurally similar amino acids for their transport or for degradation by the same enzyme. Such enzymes are exemplified by the ThDP-dependent dehydrogenases of 2-oxoadipate and branched-chain 2-oxo acids. The former transforms a common intermediate of the degradation of lysine and tryptophan, whereas the latter participates in catabolism of leucine, isoleucine, and valine. Due to the intersections in the amino acid metabolism, its organization requires a certain balance in the abundances of amino acids, which is manifested in their correlated levels. In general, the correlation coefficients are statistical indicators of dependence of two random values, and they do not point to a causal relationship between these values, i.e. a relationship where one phenomenon is the cause, necessarily leading to another phenomenon, a consequence. For example, the highly correlated levels of glutamate and alanine in the cortex (Fig. 4a) do not imply that the level of alanine necessarily determines the level of glutamate, or *vice versa*. However, in the system of our consideration, the relationships between amino acids are known to be mediated by a metabolic network. Therefore, the changed correlations are indicative of changes in this network. For example, appearance of high correlation between alanine and glutamate levels in the cerebellum of animals after the thiamine administration (Fig. 4b) is accompanied by a rearrangement of the metabolic network, particularly of the enzymes associated with transformations of these amino acids (Figs. 2 and 3). Thus, the observed change in the correlation coefficients of alanine and glutamate (Fig. 4b) could be an indicator of such rearrangement, even if all specific components of the rearrangement are not known. Unlike the correlation analysis, understanding the cause-and-consequence relationship requires more specific knowledge about the system.

Obviously, a degree of metabolic interactions between the amino acids is linked to metabolic fluxes. First, the fluxes are specific for different tissues, and that explains different correlations between amino acids in the various brain regions and in the blood plasma (Table 3). Moreover, the fluxes may vary due to external factors. For example, after the injection of thiamine, which is a regulator of central metabolism, statistically significant changes are observed in correlations between amino acids not only in the cortex and plasma, where thiamine reduces the levels of many amino acids, but also in the cerebellum, where multiple changes in the amino acid levels do not occur. Significant increase in the total and average correlation coefficients in the blood plasma and cerebellum after the thiamine injection (Table 3) manifests increasing metabolic interactions between amino acids. In other words, this increased interdependence of the amino acids means that the thiamine treatment induces a sensitization of the whole metabolic network to a change in a single amino acid. The result is consistent with published data on the thiamine regulation of the mTOR system, which is a known sensor of the availabili-

ty of amino acids [43]. Thus, correlation analysis is an additional tool to detect metabolic changes, which may be more sensitive than the analysis of the average levels of metabolites.

CONCLUSION

Our study shows that a single high dose of thiamine administered to rats causes long-term changes not only in the ThDP-dependent dehydrogenases of dicarboxylic 2-oxo acids, but also in the functionally associated enzymes. The regulation by thiamine results in a complex metabolic rearrangement that limits amino acid degradation. Restricted degradation is consistent with the observation of decreased levels and/or changed metabolic interactions of amino acids in the blood plasma. Conditions for differential assays of the dehydrogenases of 2-oxoadipate and 2-oxoglutarate in brain homogenates have been found in this study, and these are useful to detect differences in the role and regulation of these enzymes *in vivo*. Thus, this study provides a necessary basis for further characterization of the biological role of the novel ThDP-dependent dehydrogenase of 2-oxoadipate.

Acknowledgments

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