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Combined Administration of Metformin and Amprolium to Rats Affects Metabolism of Free Amino Acids in the Brain, Altering Behavior, and Heart Rate

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Abstract—The risk of developing diabetes and cardiometabolic disorders is associated with increased levels of alpha-aminoadipic acid and disturbances in the metabolism of branched-chain amino acids. The side effects of the widely used antidiabetic drug metformin include impaired degradation of branched-chain amino acids and inhibition of intracellular thiamin transport. These effects may be interconnected, as thiamine deficiency impairs the functioning of thiamine diphosphate (ThDP)-dependent dehydrogenases of 2-oxo acids involved in amino acids degradation, while diabetes is often associated with perturbed thiamine status. In this work, we investigate the action of metformin in rats with impaired thiamine availability. The reduction in the thiamine influx is induced by simultaneous administration of the thiamine transporters inhibitors metformin and amprolium. After 24 days of combined metformin/amprolium administration, no significant changes in the total brain levels of ThDP or activities of ThDP-dependent enzymes of central metabolism are observed, but the affinities of transketolase and 2-oxoglutarate dehydrogenase to ThDP increase. The treatment also significantly elevates the brain levels of free amino acids and ammonia, reduces the antioxidant defense, and alters the sympathetic/parasympathetic regulation, which is evident from changes in the ECG and behavioral parameters. Strong positive correlations between brain ThDP levels and contents of ammonia, glutathione disulfide, alpha-aminoadipate, glycine, citrulline, and ethanolamine are observed in the metformin/amprolium-treated rats, but not in the control animals. Analysis of the obtained data points to a switch in the metabolic impact of ThDP from the antioxidant and nitrogen-sparing in the control rats to the pro-oxidant and hyperammonemic in the metformin/amprolium-treated rats. As a result, metformin administration along with the amprolium-reduced thiamine supply significantly perturb the metabolism of amino acids in the rat brain, altering behavioral and ECG parameters.

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INTRODUCTION

Obesity and insulin resistance have long been known to be accompanied by disturbances in the amino acid metabolism and changes in the circulating levels of amino acids [1, 2]. In particular, diabetes is associated with the elevated plasma content of alpha-aminoadipate [3]. The therapeutic potential of the antidiabetic drug metformin can be increased by overcoming the metformin-induced impairments in the degradation of branched-chain amino acids (BCAAs) mediated by the AMP-activated protein kinase (AMPK) [4, 5].

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Amino acids are degraded through the mitochondrial tricarboxylic acid (TCA) cycle, mostly after their transamination to pyruvate, 2-oxoglutarate, or branched-chain 2-oxo acids and further oxidation by the corresponding thiamine diphosphate (ThDP)-dependent dehydrogenase complexes. An insufficiency of glucose oxidation typically causes upregulation of the amino acid degradation as an alternative energy source.

ThDP is a diphosphorylated derivative of thiamine (vitamin B1). Because ThDP acts as a coenzyme in the amino acid degradation pathways, thiamine deficiency perturbs the metabolism of amino acids. In particular, the blockade of ThDP biosynthesis and, therefore, impaired catalysis by ThDP-dependent enzymes, are known to cause changes in the levels of major amino acids in the brain of experimental animals [6, 7]. On the other hand, improvement of brain metabolism as a result of thiamine administration is accompanied by decreased oxidation of amino acids [8].

Although it has long been believed that thiamine deficiency is no more a problem in developed countries, the number of cases in which pathogenesis of neurodegenerative disorders is associated with overlooking the patient's thiamine status is increasing [9]. Thiamine consumption is not recommended together with administration of the antidiabetic drug metformin because thiamine and metformin compete for the same intracellular transporters. Owing to this, thiamine may decrease pharmacological effect of metformin. However, the opposite possibility, i.e., that the competition of metformin and thiamine for the same transporters can also decrease intracellular levels of thiamine, is considered surprisingly rarely when metformin is prescribed. This is even more surprising because the patients with type 2 diabetes mellitus repeatedly exhibit an impaired thiamine status associated with the reduced absorption and intracellular transport of thiamine [10]. Nevertheless, to our best knowledge, the association between the thiamine deficiency and metformin administration has not been studied. That said, such well-known effect of thiamine deficiency as lactic acidosis is also a common side effect of metformin therapy. Moreover, some case studies have demonstrated that thiamine alleviates the metformin-induced lactic acidosis [11, 12]. Thiamine deficiency is also known to impair oxidation of the branched-chain 2-oxoacids [6], that may underlie the impairment of the branched-chain amino acids (BCAA) degradation by metformin [4]. However, similar to lactic acidosis, this side effect of metformin has not been linked to the drug-induced thiamine deficiency either.

Abbreviations: OGDC, 2-oxoglutarate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; ThDP, thiamine diphosphate; TKT, transketolase.

Here, we study the effects of chronic metformin administration to rats whose thiamine status is simultaneously challenged by administration of amprolium. Amprolium is a coccidiostat used in poultry production. It blocks thiamine absorption in the intestine, as well as reduces its flux across the blood-brain barrier and intracellular transport [13-15]. Due to these properties, the drug is also used in basic research to create animal models of thiamine deficiency (reviewed in [16]). Amprolium administration can induce thiamine-responsive cerebrocortical necrosis and polioencephalomalacia in farm and domestic animals [17-21], thus questioning the safety of using amprolium-treated animals for human consumption [22]. Chronic amprolium administration to mice induces behavioral changes along with impaired cellular functioning [23]. Therefore, by using a combined administration of metformin and amprolium to rats, we model the comorbidities typical for diabetic patients, such as side effects of biguanide antidiabetics and undiagnosed thiamine deficiency. Taking into account that metformin and amprolium affect intracellular thiamine transporters of the SLC19A and OCT families [24-26], we compare the thiamine-dependent metabolism in the control and treated rats via (i) assessment of the levels of ThDP (the coenzyme form of thiamine), activities of ThDP-dependent enzymes, and saturation of these enzymes with ThDP in the brain homogenates, (ii) quantification of amino acids and related compounds in brain extracts, and (iii) behavioral tests and electrocardiogram (ECG) recordings in the course of the experiment. We show that by the end of the experiment, the control and experimental animals differ in the saturation of ThDP-dependent enzymes with ThDP and in the metabolic impact of ThDP and ThDP-dependent enzymes, but not in the total levels of ThDP. The drugs increase the content and strengthen the metabolic interactions of free amino acids and ammonia in the rat cerebral cortex, thus affecting animal behavior and ECG parameters.

MATERIALS AND METHODS

Animal experiments. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the European Union Directives 86/609/EEC and 2010/63/EU). Twenty-six Wistar male rats were randomly assigned to the control and experimental groups. The 5 to 6 weeks old rats entered the experiment with the body weight of 155.3 ± 2.7 and 155.4 ± 3.3 g in the control and experimental groups, respectively. At the end of the experiment, the 9 to 10 weeks old rats weighted 271.0 ± 5.1 and 278.0.3 ± 5.4 g in the control and experimental groups, respectively. Amprolium (40 mg/kg body weight) and metformin (200 mg/kg body weight

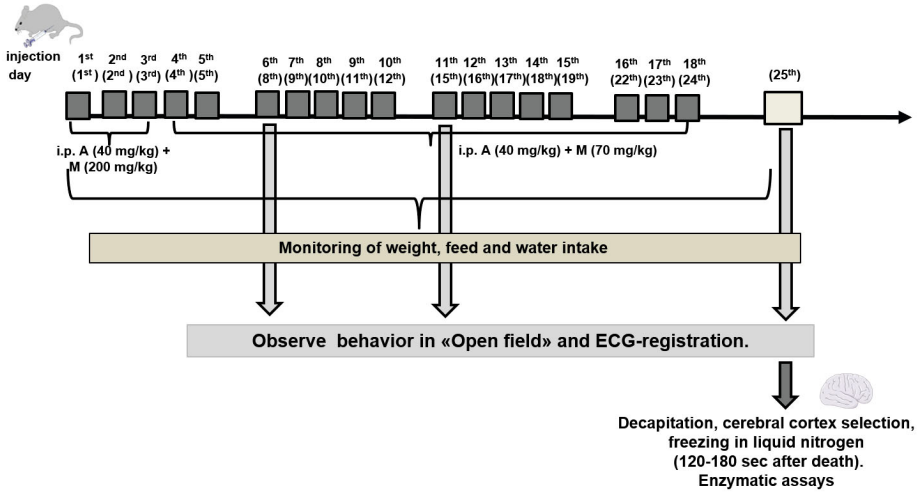

Fig. 1. The flowchart of the animal experiment. Over the period of 24 days, the drugs metformin (M) and amprolium (A) were injected on the days indicated. The first three injections of 200 mg/kg body weight metformin were followed by 15 injections of 70 mg/kg body weight metformin. Each of the metformin injections was accompanied by the second injection of 40 mg/kg body weight amprolium. Physiological and biochemical tests were performed at the indicated days as described in Materials and Methods.

for the first three injections and 70 mg/kg body weight for the next fifteen injections) were administered to rats as two separate injections (n = 14; two rats died on day 3), while the control group (n = 12) received two separate injections of physiological saline. The animals were injected in the morning (ZT 2 ± 1). Total 18 double injections were administered during 24 days according to the scheme shown in Fig. 1, i.e., 5 days with the injections were followed by the two days pause until the last three days with the injections. The doses were selected based on the published data [23, 27]. Animal weight and consumption of food and water were monitored daily. No significant differences between the experimental and control groups were observed. The open field test (OpenScience, Moscow, Russia) was used to assess the spontaneous activity of animals in an unfamiliar environment [28]. The test was conducted for three minutes in complete silence; the area was illuminated with a 15-W red lamp as described before [29, 30]. The following parameters were estimated: locomotor activity (the number of line crossings); number of entries to the central zone (the number of movements to the central zone intersecting the outer and inner circles); number of rearing (the number of stands on the hind limbs); the time and number of grooming acts, freezing time, defecation acts. Exploratory activity and anxiety were also quantified by cumulative indexes. The index of exploratory activity summarized the numbers of rearings and entries to the central zone. The index of anxiety summarized the acts of grooming and defecation and the grooming and freezing times.

The ECG was recorded for 3 min using non-invasive electrodes as described earlier [29]. The autonomic regulation of the heart was assessed from the following ECG parameters: an average R-R interval (ms); the range of R-R interval values, i.e., the difference between the maximal and minimal R-R values (dX, ms); root mean square of successive differences in the R-R intervals (RMSSD, ms); and stress index (SI). Physiological monitoring was carried out on days 8, 15, and 25 of the experiment.

After physiological tests had been conducted on day 25, the animals were sacrificed by decapitation. The brains were excised and transferred on ice; cerebral cortices were separated and frozen in liquid nitrogen within 60-90 s after decapitation.

Preparation of brain homogenates. Frozen cortex tissue was homogenized in 50 mM MOPS buffer (pH 7.0) containing 0.2 mM EGTA, 1 mM DTT, 20% glycerol, and the protease inhibitors (1 mM AEBSF, 0.8 μM aprotinin, 50 μM bestatin, 10 μM pepstatin A, 15 μM E-64, and 20 μM leupeptin) using a T10 Basic Ultra-Turrax disperser (IKA, Germany) as described before [29]. One ml of the buffer was used per 0.4 g of the tissue fresh weight (gFW). The tissue was further disrupted by sonication (7 cycles of 30-s sonication in a low-intensity mode with 30-s pauses) with a Bioruptor sonicator (Diagenode, Belgium) in an ice-cold water bath. The resulting homogenates were mixed at a 3 : 1 (v/v) ratio with the solubilization buffer containing 40 mM Tris-HCl (pH 7.4), 600 mM NaCl, 4 mM EDTA, 1% sodium deoxycholate and 4% NP-40, and incubated for at least 30 min before the assays.

Enzyme assays. The activity of transketolase (TKT) was assessed spectrophotometrically from the rate of NADH oxidation in the triosephosphate isomerase/glycerol-3-phosphate dehydrogenase coupled system by the established method [31] modified to use the microplate format [32]. The linear part of the product

accumulation curve (30 min) was used to calculate the reaction rate followed by subtraction of the background reaction rate assayed without pentose phosphates. The activity of pyruvate dehydrogenase complex (PDC) was determined calorimetrically on a CLARIOstar Plus microplate reader (BMG Labtech, Germany) from the NADH production coupled to iodonitrotetrazolium reduction to formazan [33] with modifications described earlier [34, 35]. The linear part of the product accumulation curve from the 1st to 10th min was used to calculate the reaction rate. The activity of the 2-oxoglutarate dehydrogenase complex (OGDC) was assessed from the absorbance of produced NADH at 340 nm as described before [8] using a Sunrise microplate reader (Tecan, Austria). A steady-state reaction rate from the 5th to 10th min, i.e., after completion of a lag period, was used to calculate the activity. To assess the activities of endogenous holoenzymes of the ThDP-dependent dehydrogenases, the assays were conducted in the absence of $MgCl_2$ and ThDP in the reaction media. The endogenous holotransketolase was determined in the medium lacking ThDP.

Quantification of brain metabolites. Free amino and related amino compounds were quantified by ion-exchange chromatography with ninhydrin derivatization in the methanol-acetic acid extracts of the brain cortex as described before [36]. Taurine was quantified within a non-resolved peak with phosphoethanolamine (PEA). In several studies on mammalian brain, relative abundances of taurine and PEA vary from comparable to up to 10 times more abundant taurine than PEA [37-40]. Oxidized glutathione and NAD^+ were measured with a fluorometric assay [41, 42] in black 96-well microplates using a CLARIOstar Plus microplate reader. ThDP was quantified with the apo-TKT activation assay [43] modified for the microplate format [44].

Statistical analysis was performed with the STATISTICA (version 6.0), GraphPad Prism (version 8.4), and R (version 4.3) software packages. The outliers were identified with the iterative Grubbs' test at Alpha set to 0.01, and excluded from statistical analysis. The differences in the content of metabolites between two groups were analyzed with the *t*-test in view of the normal data distribution according to D'Agostino–Pearson test. The differences in cumulative parameters of correlations between metabolites (sum and mean correlation coefficients as well as the numbers of significant positive and negative correlations) were analyzed by Mann–Whitney test due to non-normal distribution. When more than two groups were analyzed, factorial analysis of variance (ANOVA) was used together with the *post-hoc* comparison of group averages by the Šídák test. The data were presented as mean ± standard error of mean (SEM). The correlations between the assayed parameters were analyzed using the Spearman's rank correlation coefficient, as not all physiological parameter values were normally distributed. The differences between the groups and correlations were considered significant at $p \le 0.05$; the values of $p \le 0.1$ were considered as trends.

RESULTS

Levels of ThDP, activities of ThDP-dependent enzymes and redox-related compounds as indicators of thiamine-dependent metabolism in the rat cerebral cortex. The content of ThDP may characterize potential changes in the thiamine-dependent metabolism after combined chronic administration of the thiamine transport inhibitors metformin and amprolium, as ThDP is a coenzyme form and major derivative of thiamine in the brain. In view of the essential role of ThDP in the central redox metabolism, the levels of NAD^+, as well as those of the antioxidant peptides carnosine and glutathione, are other important indicators of the thiamine-dependent metabolic changes.

As seen from Fig. 2, a minor decrease in the total ThDP content in the brain after the treatment does not reach the level of statistical significance ($p = 0.28$). However, administration of thiamine transport inhibitors causes a certain level of oxidative stress, which is a well-known feature of thiamine deficiency [45, 46]. The oxidative stress is evidenced by a decrease in the content of carnosine, which protects the brain from the damage by peroxynitrite [47], and a trend ($p = 0.09$) to elevation of oxidized glutathione (GSSG) (Fig. 2).

The total activities of ThDP-dependent enzymes in the brain cortex, which are determined in the presence of ThDP in the assay media, do not differ in the control and treated groups. However, the extent of enzyme activation by ThDP added to the assay medium decreases after the treatment. For TKT, this is manifested as a disappearance in the treated samples of statistically significant, although small, activation of TKT by ThDP that is observed in the control samples (Fig. 3a, upper panel). For OGDC, there is a statistically significant decrease in the apo-OGDC fraction in the treated vs. control samples (Fig. 3c, bottom panel). Activation of PDC by ThDP is ~100% in both the control and treated groups, which is in agreement with the known requirement for ThDP addition to the PDC assay medium [34, 48]. In contrast to TKT and OGDC, the dissociation of PDC holoenzyme under the assay conditions is complete in both the control and treated groups, which does not allow one to detect changes in the enzyme saturation with ThDP, which may have been induced by the treatment. However, for both TKT and OGDC, the levels of enzyme saturation with ThDP increase after the treatment with metformin/amprolium, compared to the control group.

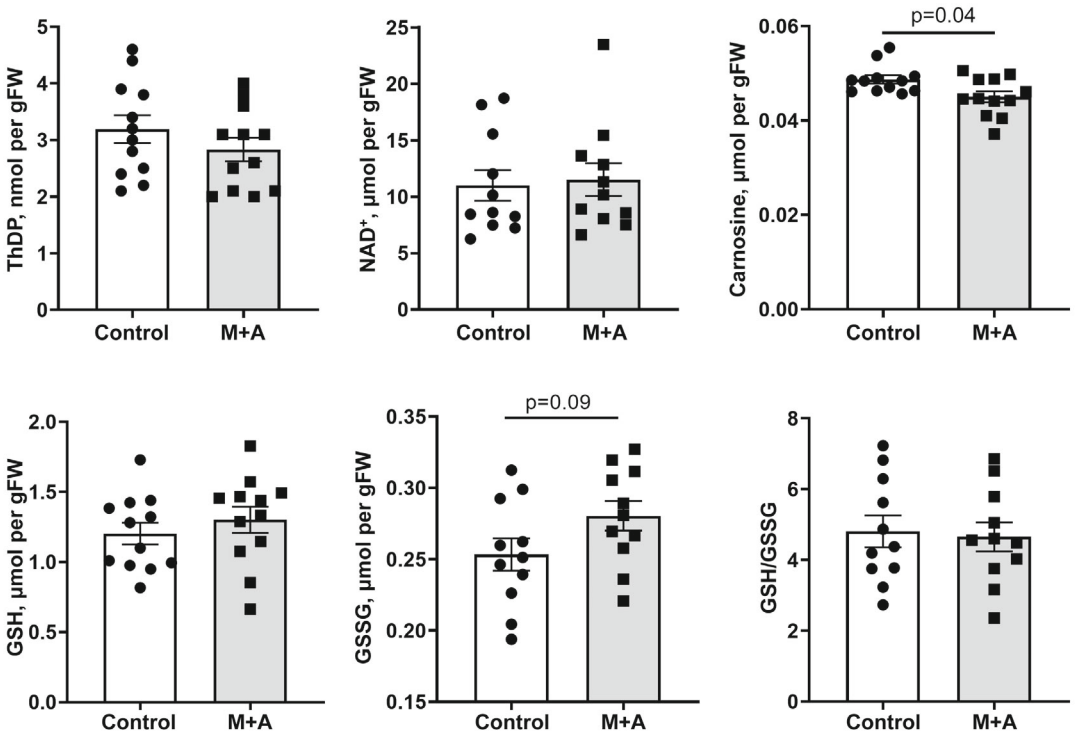

Fig. 2. The levels of ThDP, NAD^+, antioxidant peptides carnosine and glutathione (GSH), oxidized glutathione (GSSG) and the ratio of the reduced and oxidized glutathione in the cerebral cortex of rats treated with metformin/amprolium (M+A) and control animals. gFW, g of fresh weight.

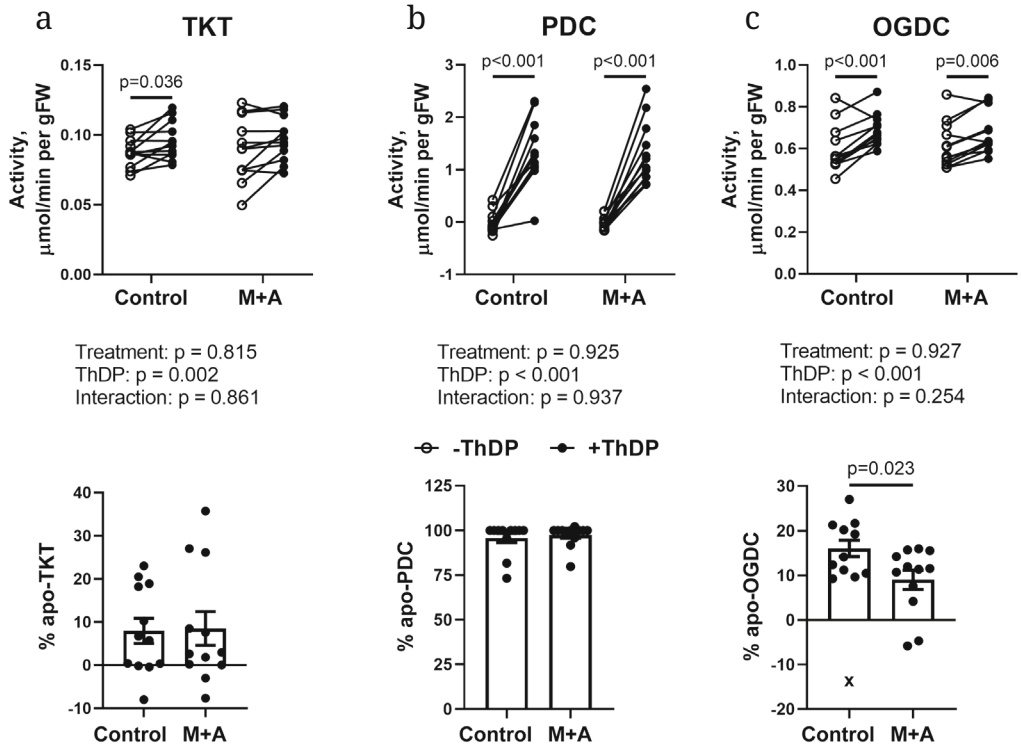

Fig. 3. Activation of TKT (a), PDC (b), and OGDC (c) by ThDP addition to the assay medium in the brain cortices of the control rats and rats treated with metformin/amprolium (M+A). In the upper panel, open and solid points indicate activities measured in the absence and presence of ThDP, respectively (analysis by repeated measures ANOVA). Bottom panel compares the fractions of endogenous TKT, PDC, and OGDC apoenzymes in the treated and control samples, calculated as [1 – (Activity without ThDP)/(Activity with ThDP)] × 100%. The outlier excluded from analysis is indicated with "x".

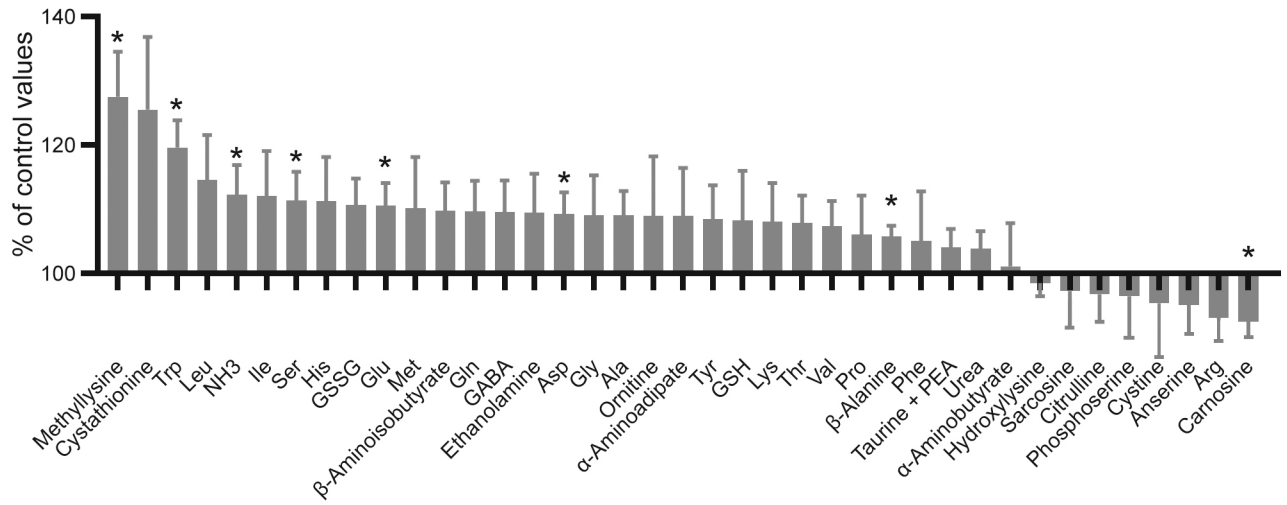

Fig. 4. Changes (%) in the content of amino acids and related compounds in the cerebral cortex of rats treated with metformin/amprolium vs. control animals (n = 12 in each group). The outliers outside of y-axis range (not shown) are excluded from statistical analysis; * significant difference from the control values set as 100%.

If changes in the ThDP-induced activation of TKT and OGDC were due to different ThDP contents in the rat brains, the points of the endogenous holoenzyme activities or the enzyme activation by ThDP vs. ThDP level in the brains of the treated and non-treated animals would have occupied different regions of the XY space. If changes in the enzyme activation by ThDP were due to changes in the enzyme properties, the points would have occupied the same XY space. The correlations between the ThDP level and contents of endogenous holo- or apoenzymes (Fig. S1 in the Online Resource 1) favor the second rather than the first assumption, as the data for both animal groups occupy the same XY space in the graphs. Remarkably, apo-TKT in the control rats demonstrates an expected statistically significant negative correlation with the ThDP content in the brain. Alteration of this correlation in the treated animals provides additional support for the treatment-changed affinity of TKT to ThDP. The activation of mitochondrial OGDC by ThDP added to the reaction medium shows no significant correlation with the total tissue level of ThDP in either treated or control animals.

As a result, the treatment with metformin/amprolium does not significantly decrease the total brain levels of ThDP, but increases the affinities of TKT and OGDC to the coenzyme ThDP in the rat brain.

Changes in the profiles of free amino acids and related compounds in the rat brain after chronic administration of metformin and amprolium. Compared to the control group, chronic administration of metformin/amprolium induces statistically significant increases in the content of methyllysine, tryptophan, serine, glutamate, aspartate, and beta-alanine, decreasing the content of carnosine (Fig. 4). There are also trends ($0.05 < p \le 0.1$) toward increases in the levels of cystathionine, leucine, beta-aminoisobutyrate and alanine. The content of free brain amino acids, including BCAAs, mostly increases. This is accompanied by a statistically significant elevation in the brain ammonia level (Fig. 4), which suggests hyperammonemia resulting from the elevated degradation of accumulating amino acids. Therefore, in addition to the perturbed levels of the peptides involved in cell antioxidant defense, i.e., carnosine and oxidized glutathione (Fig. 2), the overall perturbation of amino acid metabolism, associated with hyperammonemia, represents a marker of pathological changes in the brain of the metformin/amprolium-treated rats.

Analysis of correlations between the levels of ThDP or activities of ThDP-dependent enzymes and the content of brain metabolites. The average tissue levels of ThDP or activities of ThDP-dependent enzymes (Figs. 2 and 3) provide a rough measure of changes in the thiamine status, but are not suitable to resolve the differences in metabolic fluxes in the control and treated states. In addition to the mean values of the parameters, pairwise correlations between the thiamine status parameters and the content of metabolites of the ThDP-dependent network are useful to characterize the treatment-induced metabolic changes. As shown in Table 1, the treatment strongly affects significance of the correlations between the ThDP content or activities of ThDP-dependent enzymes and the levels of redox indicators or amino acids. In particular, the treatment induces significant positive correlations between the levels of ThDP and pathological markers, such as levels of glutathione disulfide, α-aminoadipate and ammonia. Besides, ThDP levels in the brains of the treated rats become positively correlated with the contents of citrulline, ethanolamine, glycine, and combined level of taurine and phosphoethanolamine.

Table 1. The correlations between the levels of ThDP or activities of ThDP-dependent enzymes and the contents of NAD^+, antioxidant peptides, free amino acids or related metabolites in the cerebral cortex of the control rats (Ctrl) and rats treated with metformin/amprolium (M+A)

Parameter	ThDP		PDC –ThDP		PDC +ThDP		OGDC –ThDP		OGDC +ThDP		TK –ThDP		TK +ThDP	
	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A
NAD^+	0.34	–0.47	–0.21	–0.11	–0.05	0.10	**0.66**	**–0.70**	**0.77**	**–0.60**	0.36	0.30	–0.15	0.55
Carnosine	0.29	–0.33	–0.31	0.33	0.12	0.50	–0.08	0.08	0.21	0.38	0.27	**–0.62**	–0.07	–0.43
GSH	0.06	–0.07	–0.16	–0.01	**–0.73**	0.10	0.27	–0.13	–0.03	–0.01	–0.18	0.02	–0.45	0.15
GSSG	–0.05	**0.76**	0.38	–0.15	0.25	**–0.60**	–0.02	0.38	0.08	0.26	–0.03	0.31	0.11	0.11
GSH/GSSG	–0.14	–0.54	–0.17	–0.16	–0.04	0.46	–0.11	**–0.70**	–0.11	**–0.61**	0.00	0.19	–0.23	0.38
GSH+2*GSSG	–0.04	0.36	0.14	–0.52	0.02	–0.28	0.04	–0.27	0.16	–0.24	–0.16	**0.70**	–0.39	**0.69**
α-Aminoadipate	–0.18	**0.75**	0.08	–0.01	0.10	**–0.61**	0.36	**0.61**	0.16	0.45	–0.07	0.15	–0.12	–0.01
α-Aminobutyrate	0.15	0.01	–0.08	**–0.57**	–0.08	–0.28	0.34	–0.31	0.20	–0.45	0.09	**0.59**	–0.03	**0.71**
Arg	–0.45	0.26	0.31	0.37	0.29	0.11	**–0.65**	–0.05	–0.45	0.04	0.17	–0.10	0.34	0.01
β-Aminoisobutyrate	0.07	0.37	–0.12	**–0.57**	**–0.69**	**–0.58**	0.10	0.32	–0.30	0.06	–0.28	0.34	–0.36	0.21
Citrulline	–0.16	**0.60**	0.21	–0.10	–0.07	–0.36	0.50	–0.03	0.52	–0.20	–0.04	0.31	–0.28	0.22
Cystathionine	0.31	–0.17	–0.10	**0.75**	–0.15	0.45	–0.21	–0.27	–0.08	–0.09	0.47	–0.36	0.29	–0.26
Ethanolamine	0.52	**0.60**	–0.22	0.06	0.00	–0.31	0.38	0.26	0.51	0.41	0.35	0.23	–0.03	0.22
Gly	–0.24	**0.57**	0.01	0.07	0.42	–0.06	–0.29	0.01	–0.11	0.24	0.37	0.08	0.55	0.15
Hydroxylysine	0.11	0.30	–0.08	0.26	0.11	0.40	**–0.60**	0.06	–0.17	0.27	0.48	–0.49	0.37	–0.48
Ile	0.02	0.57	–0.03	0.15	0.35	–0.24	–0.54	0.01	–0.03	0.11	**0.73**	0.14	**0.73**	0.21
Leu	–0.13	0.52	0.07	0.21	0.52	–0.20	**–0.61**	0.04	–0.13	0.19	**0.58**	0.10	**0.69**	0.18
Lys	**0.76**	0.53	**–0.65**	0.02	–0.21	–0.11	–0.04	0.11	0.27	0.32	0.52	0.03	0.10	0.06
Met	–0.19	0.47	0.08	0.20	0.43	0.10	**–0.76**	–0.22	–0.23	–0.03	**0.59**	–0.02	**0.65**	–0.02
NH3	0.19	**0.66**	–0.01	–0.01	–0.26	–0.26	–0.37	0.08	–0.49	0.31	0.20	0.30	0.40	0.29
Phe	–0.47	0.43	0.44	0.27	**0.57**	0.21	**–0.57**	0.01	–0.32	0.30	0.15	–0.24	0.50	–0.19
Phosphoserine	0.10	0.31	–0.15	–0.55	0.29	–0.22	0.33	**0.59**	**0.61**	**0.76**	0.32	0.08	0.07	–0.03
Taurine+PEA	0.19	**0.79**	–0.22	–0.31	–0.29	–0.48	0.34	0.42	0.33	0.38	0.30	0.34	–0.13	0.00
Thr	**0.57**	0.49	–0.38	–0.13	0.01	0.06	–0.24	–0.19	0.03	0.18	**0.88**	0.20	**0.64**	0.29
Trp	**–0.79**	–0.33	0.73	**0.56**	0.20	0.25	–0.49	**–0.59**	**–0.66**	–0.36	–0.08	0.03	0.35	0.29
Urea	0.33	0.43	–0.15	0.17	0.24	–0.13	0.07	–0.25	0.35	–0.02	**0.63**	0.46	0.28	0.54
Val	–0.21	0.44	0.28	0.27	**0.70**	–0.02	–0.20	–0.19	0.17	0.12	0.56	0.15	**0.67**	0.29

Note. The table shows metabolites whose contents demonstrate at least one significant correlation with the levels of ThDP or activities of ThDP-dependent enzymes. Each cell in the table shows the Spearman's rank correlation coefficient for a given pair of parameters. Coefficients of the correlations with changed level of statistical significance in the control and treated states are shown in red on grey background. Coefficients of significant correlations ($p \le 0.05$) are given in bold.

In contrast, significances of the positive correlation between the levels of ThDP and lysine, and the negative correlation between the levels of ThDP and tryptophan in the control animals are reduced by the treatment. Taken together, these data point to changed metabolic contribution of ThDP to the cellular redox state and metabolism of amino acids after chronic administration of thiamine transport inhibitors.

Similarly, significances of the correlations between the brain activities of ThDP-dependent enzymes and the levels of redox indicators or amino acids are strongly changed by the treatment (Table 1). In particular, after the treatment, the OGDC activity correlates negatively with the levels of NAD^+ and glutathione redox state (GSH/GSSG), while in the control animals the OGDC activity exhibits a strong positive correlation with the NAD^+ content. Unlike the brain TKT activity of the control rats, the brain TKT activity of the treated rats correlates positively with the levels of total glutathione (GSH+2*GSSG) and α-aminobutyrate, and negatively with the level of carnosine. In addition, significant positive correlations of the TKT activity with the BCAA levels, observed in the control group, disappear after the treatment. Overall, correlation analysis indicates that the metabolic relationships between ThDP or ThDP-dependent enzymes and free amino acids or related compounds are different in the treated and control rat brain.

The treatment effects on the rat behavior in the open field test and ECG. Physiological tests during the experiment help understanding how the observed changes in the metabolism of brain amino acids affect animal behavior and ECG. Unlike the biochemical characterization of the brain tissue, physiological testing may be repeated in the course of experiment, thus providing insights into the development of treatment-induced changes. According to ANOVA, the factor "treatment" by metformin/amprolium is significant for the number of entries to the central zone (Fig. 5a) and associated parameter of locomotor activity (Fig. 5b). The cumulative indexes of exploratory activity and anxiety also show the significance of the "treatment" factor (Fig. 5b). Significant interaction between the number of entries to the central zone and days of treatment (Fig. 5a) manifests in an increase in this parameter after the day 15 in the treated rats only. As a result, on the day 25, the number of entries to the central zone for the treated animals is significantly higher than that for the control ones. Obviously, this difference contributes the most to a higher cumulative index of exploratory activity in the treated vs. control rats. The overall significances of the treatment for the locomotor activity ($p = 0.046$) and cumulative anxiety index ($p = 0.03$) do not reveal particular differences between the groups tested on specific days of the treatment (Fig. 5b).

In view of the fact that the treatment does not affect the number of rearings, but is significant for the number of entries to the central zone ($p = 0.02$) and the locomotor activity ($p = 0.046$), the observed increase in the number of entries to the central zone and accompanying increase in exploratory activity ($p = 0.02$) seem to be linked to the overall elevation of locomotor activity, induced by the treatment (Fig. 5, a, b). These behavioral changes in the treated rats are reciprocated by their increased heart rate, as the treatment induces a decrease in the R-R interval of ECG in the treated rats only (Fig. 5c).

Therefore, chronic administration of metformin and amprolium affects several behavioral and ECG parameters in a time-dependent manner. The most significant differences between the control and treated animals are observed by the end of experiment, manifested in the increased number of entries to the central zone and related cumulative index of exploratory activity in the treated vs. control rats.

Analysis of correlations between the levels of ThDP or activities of ThDP-dependent enzymes and the behavioral or ECG parameters. In order to understand how much the thiamine status and its interplay with the content of brain metabolites (Table 1) contribute to the metformin/amprolium-induced changes in rat behavior and ECG, the correlations of thiamine-dependent biochemical parameters with the physiological ones are analyzed. The results of analysis (Table 2) reveal an interesting time dependence of the treatment effects on the correlations. In the rats sacrificed on the day 25, the ThDP levels at this final day of experiment correlate more significantly with the behavioral parameters determined on the day 15 than with those determined on the day 25. That is, in the control animals the brain ThDP levels on the day 25 correlate positively with the cumulative index of anxiety on the day 15 and negatively – with the cumulative index of exploratory activity on the day 15, with both the correlations disappearing in the treated rats. Besides, in the treated rats, the brain ThDP level on the day 25 correlates negatively with locomotion on the day 15, while the correlation is absent in the control rats. Remarkably, these correlations are in accordance with physiological considerations. In the control animals, the content of ThDP is positively associated with anxiety and negatively with the exploratory activity, as exploratory activity is known to be higher when anxiety is low. Similarly, in the treated animals, the content of ThDP correlates negatively with both the locomotor activity and the number of entries to the central zone, since the two physiological parameters are known to change in a similar direction. The graphs in Fig. 5 show that on day 15, the trends observed for many assessed physiological parameters switch to the opposite: if a parameter is mostly decreasing from day 8

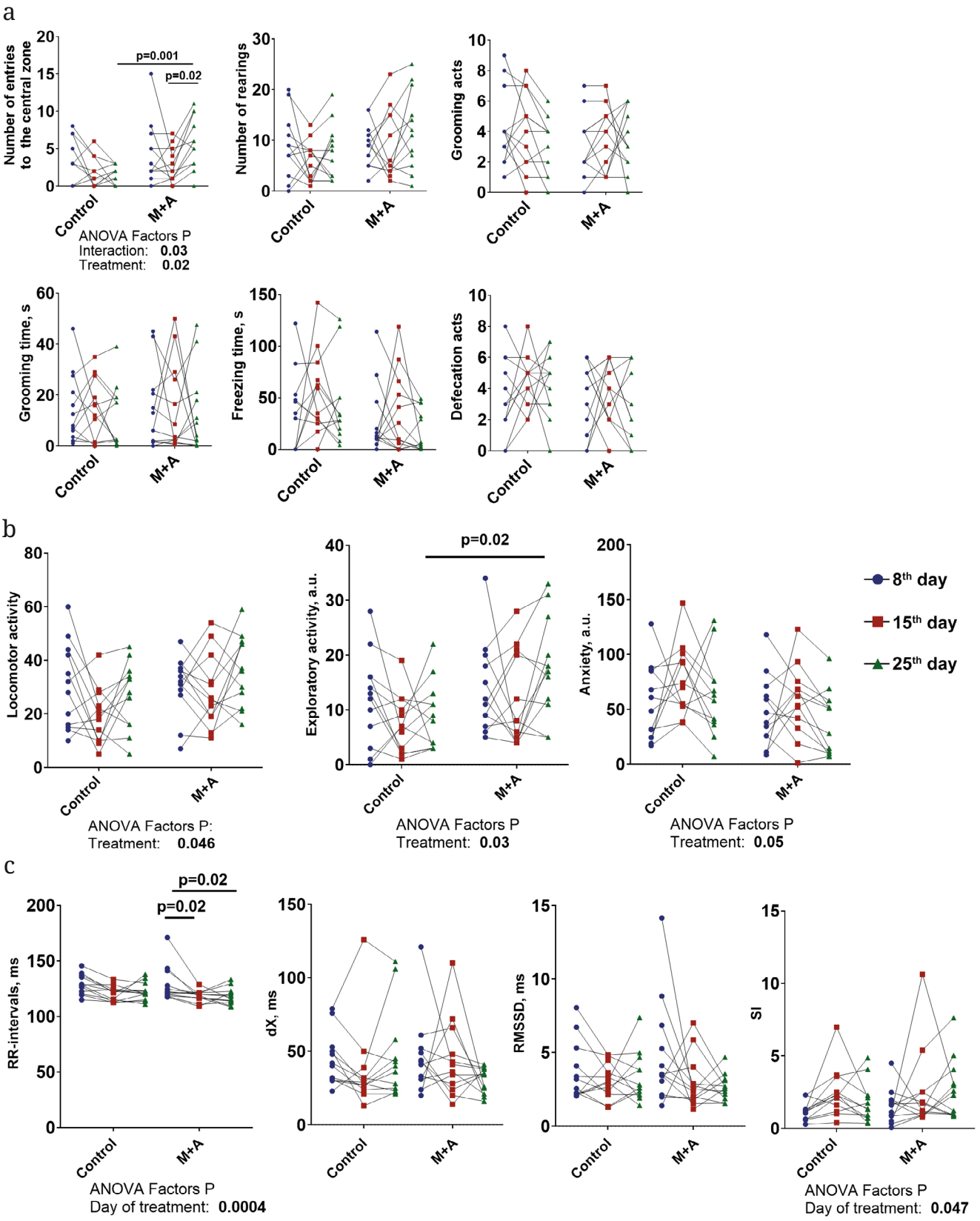

Fig. 5. Time-dependent changes in rat behavior in the open field test (a, b) and ECG parameters (c) during chronic administration of metformin/amprolium (M+A) in comparison with the control rats. Statistically significant differences between the groups are shown on the graphs; statistically significant factors, such as "treatment" by metformin/amprolium and the "treatment time", along with their interaction, are shown under the graphs (according to the repeated measures ANOVA with Šídák *post-hoc* test).

to day 15, it starts to increase from day 15 to day 25, and vice versa. This switch might be of either adaptive or maladaptive nature. The importance of ThDP levels in the observed responses is emphasized by the fact that the number of entries to the central zone is the only behavioral parameter that differs significantly on day 25

Table 2. Correlations of the brain levels of ThDP or activities of ThDP-dependent enzymes in the presence (+) or absence (–) of ThDP in the assay media with ECG of behavioral parameters in the control (Ctrl) and metformin/amprolium-treated (M+A) rats

Parameter	ThDP		PDC –ThDP		PDC +ThDP		OGDC –ThDP		OGDC +ThDP		TKT –ThDP		TKT +ThDP	
	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A	Ctrl	M+A
R-R interval	–0.24	–0.19	0.03	–0.08	**0.65**	0.06	0.02	–0.19	–0.01	–0.15	–0.22	0.13	–0.09	0.24
dX	–0.37	0.05	0.27	–0.23	0.04	–0.08	0.18	0.02	–0.10	–0.02	**–0.75**	0.48	–0.50	0.42
RMSSD	–0.10	–0.18	0.02	0.09	0.11	–0.21	0.13	0.29	0.03	0.20	–0.34	0.04	–0.35	–0.01
SI	0.36	–0.06	–0.08	0.34	–0.27	0.21	–0.06	0.03	0.21	0.03	**0.60**	–0.40	0.38	–0.43
Anxiety, day 8	0.04	–0.02	0.20	0.41	–0.52	0.02	–0.14	–0.20	–0.45	–0.30	–0.37	–0.05	–0.10	–0.06
Anxiety, day 15	**0.57**	0.02	–0.53	–0.10	0.05	0.27	0.30	–0.05	0.45	–0.01	0.29	–0.38	0.01	–0.24
Anxiety, day 25	–0.16	0.08	0.01	–0.11	0.36	0.14	0.44	0.08	0.14	0.02	**–0.59**	0.21	–0.39	0.03
Grooming time	–0.04	0.52	–0.15	–0.09	–0.14	–0.40	0.00	0.21	0.10	–0.04	–0.28	0.42	–0.30	0.01
Freezing time	–0.31	–0.19	0.28	–0.14	0.38	0.36	0.47	–0.19	0.15	–0.14	**–0.65**	0.09	–0.37	0.23
Defecation acts	–0.44	–0.11	0.14	–0.37	0.28	0.23	0.05	–0.43	–0.07	–0.01	–0.45	0.03	–0.07	0.24
Grooming acts	–0.25	–0.17	0.22	0.04	0.05	–0.05	–0.24	0.18	–0.06	–0.05	–0.27	0.23	–0.19	0.09
Exploratory activity, day 8	–0.33	–0.46	0.04	–0.28	**0.61**	0.13	–0.06	0.00	0.15	0.23	0.39	0.07	0.32	0.22
Exploratory activity, day15	**–0.60**	–0.25	0.43	–0.22	0.19	–0.13	–0.34	–0.23	–0.47	–0.22	–0.10	0.22	0.03	0.22
Exploratory activity, day 25	–0.10	–0.33	0.11	0.21	0.07	0.02	–0.43	–0.25	–0.01	–0.32	**0.58**	–0.29	0.49	–0.15
Number of rearings	–0.06	0.03	0.25	0.13	0.18	–0.27	–0.45	–0.27	0.01	–0.32	**0.63**	0.03	**0.57**	0.09
Number of entries to the central zone	–0.38	**–0.80**	0.09	0.39	0.03	0.55	–0.43	–0.10	–0.34	–0.08	0.12	**–0.69**	0.20	–0.39
Locomotor activity, day 8	–0.40	–0.52	0.05	0.20	0.41	0.31	0.08	0.04	0.22	0.16	0.15	–0.32	0.06	–0.06
Locomotor activity, day 15	–0.26	**–0.58**	0.21	0.22	–0.36	–0.02	0.02	–0.18	–0.25	–0.34	–0.18	0.16	–0.30	0.23
Locomotor activity, day 25	–0.05	0.10	0.07	0.00	–0.12	–0.30	**–0.61**	0.12	–0.24	0.06	**0.60**	–0.15	0.52	–0.18

Note. Each cell shows the Spearman's rank correlation coefficient for a given pair of parameters. Correlations whose statistical significance ($p \leq 0.05$) differ between the control and treated animals, are shown in red on grey background. Statistically significant correlation coefficients are given in bold. The correlations with "Locomotor activity" and cumulative indexes "Anxiety" or "Exploratory activity", use the data on days 8, 15, and 25; the correlations with other physiological parameters are shown only for the data on day 25.

between the treated and control animals, and at the same time, the only parameter in the treated group that correlates with the ThDP levels on day 25. Significant correlations of both the brain ThDP levels or activity of ThDP-dependent TKT with the number of entries to the central zone on day 25 (Table 2) indicate that the physiological switch on day 15, when the difference between the groups has not been yet manifested (Fig. 5), is linked to the ThDP-dependent brain metabolism.

At the end of experiment, the activities of ThDP-dependent enzymes mostly exhibit significant correlations with behavioral or ECG parameters in the control animals, with the correlations disappearing in the treated animals (Table 2). The same is observed for the correlation between the PDC activity and exploratory activity on day 8. Only the negative correlation between the TKT activity and the number of entries to the central zone arises in the treated animals, absent in the control ones.

Overall, the activity of endogenous holoenzyme of TKT shows the highest number of significant correlations, either positive or negative, with the behavioral or ECG parameters, mostly in the control animals (Table 2).

Changes in the rat brain, characterized by the treatment-modified correlations of biochemical and physiological parameters. The patterns of interaction between the contents of metabolites, enzyme activities, and physiological parameters in the control and treated rats are shown in Figs. 6 and 7, respectively.

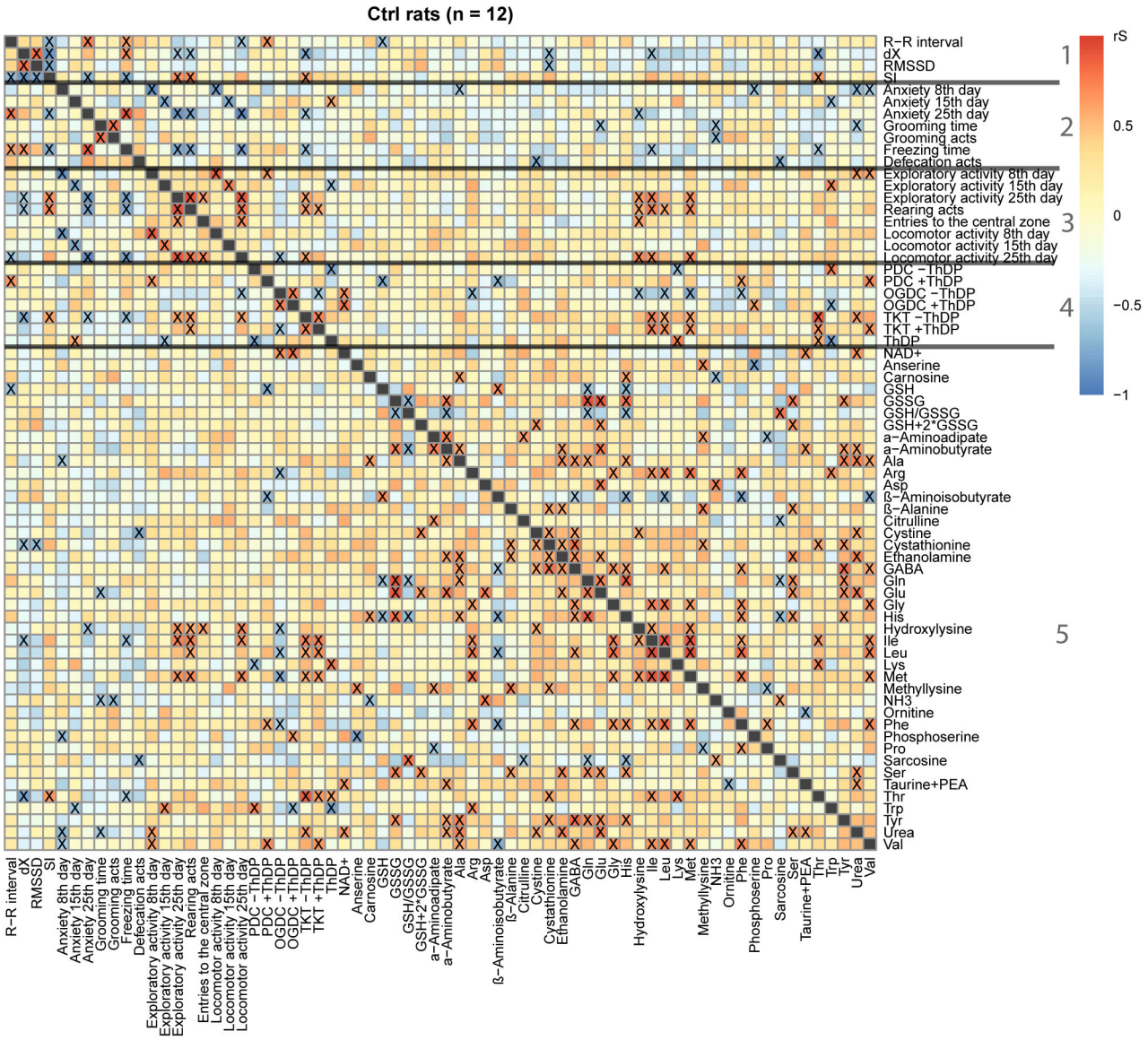

Fig. 6. Correlation matrix for physiological and biochemical parameters in the control rats. Thick lines divide the parameters into five groups: (1) ECG parameters, (2) anxiety parameters, (3) parameters of exploratory and locomotor activities, (4) activities of ThDP-dependent enzymes together with ThDP levels, and (5) levels of NAD^+, amino acids, and related compounds.

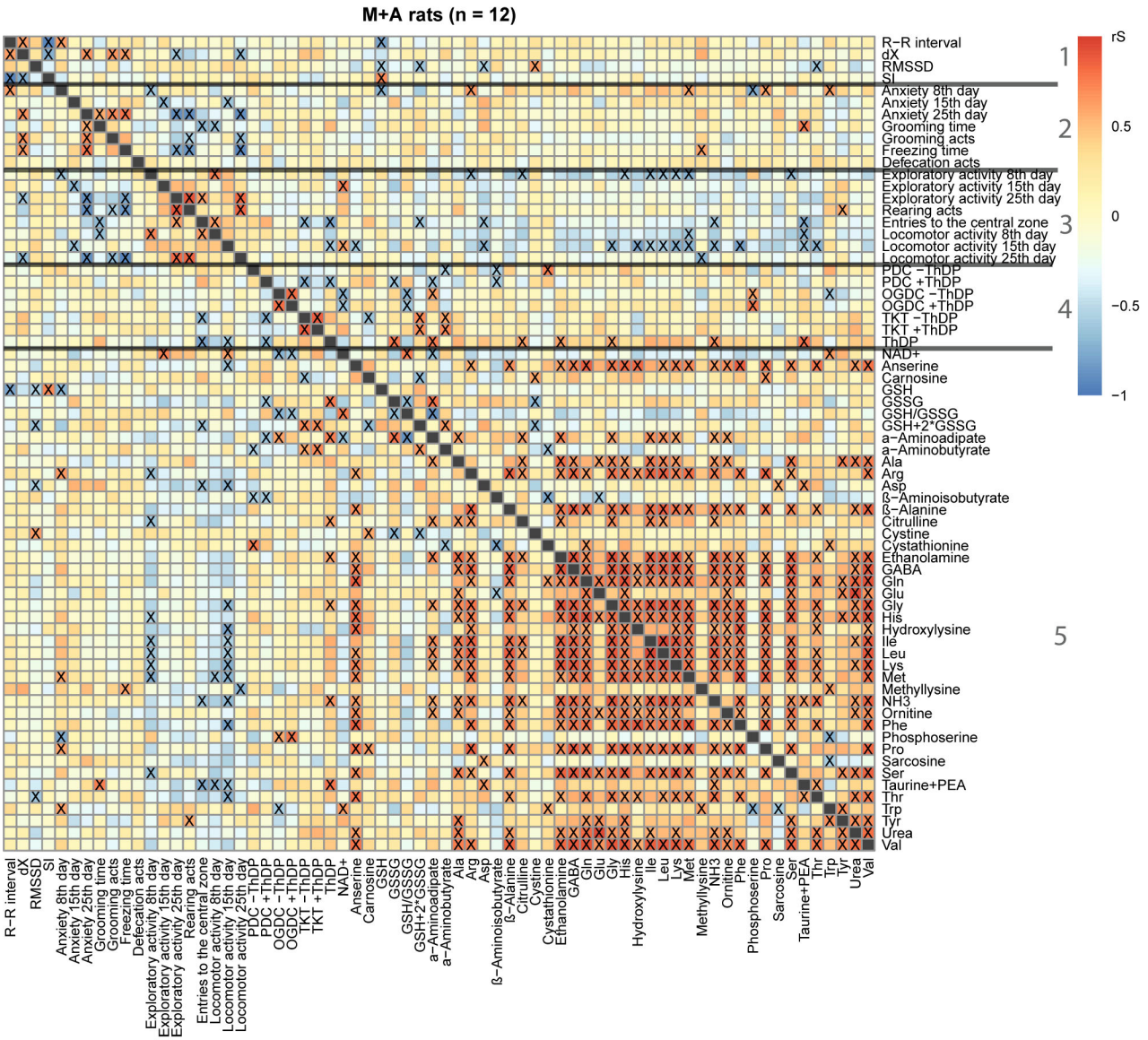

Fig. 7. Correlation matrix for physiological and biochemical parameters after chronic administration of metformin/amprolium (M+A). Thick lines divide the parameters into five groups: (1) ECG parameters, (2) anxiety parameters, (3) parameters of exploratory and locomotor activities, (4) activities of ThDP-dependent enzymes together with ThDP levels, and (5) levels of NAD^+, amino acids, and related compounds.

Overall, the correlation matrices for the control and treated animals reveal a significantly higher positive interdependence between the contents of brain amino acids and related compounds (group 5 in Figs. 6 and 7) after the treatment with metformin/amprolium. Compared to the control animals, a 2-fold increase in both the number of positive correlations and values of corresponding correlation coefficients is observed (Table S1 in the Online Resource 1). A simultaneous increase in the content of free amino acids in the brain (Fig. 4) and in the number of positive correlations between the levels of different amino acids manifests a common cause for the elevation in content of different amino acids, most probably protein degradation.

Important shifts in the correlations between the levels of metabolites and activities of ThDP-dependent enzymes, caused by the treatment (Table 1 and discussion above), are accompanied by changes in the correlations between the ThDP-linked markers of pathological states. In particular, the change in the sign of the correlation between the NAD^+ content and the OGDC activity is accompanied by changes in the significances of other NAD^+ correlations after the treatment. In the control animals, the NAD^+ levels are positively correlated to the contents of urea and combined levels of taurine (antioxidant) and phosphoethanolamine. After the treatment, these correlations are substituted by the positive correlations between the levels of NAD^+

and tryptophan or glutathione antioxidant potential (GSH/GSSG), added by a significant negative correlation between the levels of NAD^+ and α-aminoadipate. Remarkably, in the treated animals, the levels of α-aminoadipate and GSH/GSSG correlate negatively, while the levels of α-aminoadipate and GSSG correlate positively, with all these correlations absent in the control animals. Significant correlations between the levels of antioxidant carnosine and ammonia (negative), alanine (positive), or histidine (positive) are observed in the control animals. In the treated animals, these correlations are substituted by significant negative correlations between the levels of carnosine and total glutathione or activity of TKT, and by significant positive correlations between the levels of carnosine and proline or cystine. Thus, simultaneously with modified interactions of the levels of ThDP or activities of ThDP-dependent enzymes with other parameters, administration of metformin/amprolium changes the relationships involving different pathological markers, such as GSSG which is a marker of oxidative stress, or α-aminoadipate which is a marker of diabetes [3].

Regarding changes in the relationships between the biochemical and physiological parameters, the treatment with metformin/amprolium switches the sign of correlations between the contents of amino acids and behavioral parameters of the groups 2 and 3. Predominantly negative correlations between the parameters of anxiety and the contents of amino acids in the control animals become predominately positive in the treated animals. At the same time, the opposite is observed for the correlations between the amino acid contents and parameters of exploratory and locomotor activities (Figs. 6 and 7). The fact that in the different states of the rats, the relationships between the brain amino acids and behavioral parameters are different, corresponds to the non-causative nature of correlations. Nevertheless, irrespectively of the treatment, the opposite relationships between the contents of amino acids and behavioral parameters in the groups 2 and 3 are observed. This finding corresponds to a negative relation between anxiety and exploratory or locomotor activities: an anxious animal prefers neither to move, nor to explore.

The negative correlations of SI (an indicator of sympathetic activity) with the duration of the R-R intervals and their variability (dX) manifest the basic physiological relationship: The lower the sympathetic activity, the longer the R-R interval and the higher its variability. The significances of these correlations are therefore preserved after the treatment. However, the negative correlation of SI with RMSSD in the control group disappears in the treated group. Simultaneously, a positive correlation of dX with RMSSD in the control rats is substituted by the positive correlation of dX with the R-R interval. Hence, chronic administration of metformin/amprolium affects the balance between the sympathetic and parasympathetic regulation manifested in the correlations between the specific ECG parameters. These changes are accompanied by alterations in the correlations between ECG parameters and the contents of brain metabolites involved in the cellular redox regulation (reduced, oxidized and total glutathione, cystine, cystathionine, taurine+phosphoethanolamine), or some other amino acids (isoleucine, aspartate, and threonine) (Figs. 6 and 7). The negative correlation between the R-R interval and the levels of reduced glutathione is not changed by the treatment, representing the only significant correlation of the R-R interval with the contents of studied metabolites.

Thus, administration of metformin/amprolium affects the balance between the sympathetic and parasympathetic regulation, that is associated with changes in the correlations between the ECG parameters and levels of the brain amino acids. Simultaneously, the regulation of this balance is manifested as changes in the anxiety indicators. In rodents, freezing (a form of behavioral inhibition that is also an indicator of anxiety) is accompanied by a decrease in the heart rate [49]. In our control animals, this is evident from the positive correlations of freezing time with the duration of R-R intervals and dX, as well as a negative correlation of freezing time with SI. However, only the positive correlation between the freezing time and dX is significant after the treatment, further supporting the treatment-induced changes in the balance of sympathetic/parasympathetic regulation.

DISCUSSION

In this work, we show that chronic administration of the thiamine influx inhibitors metformin and amprolium perturbs the amino acid metabolism in the rat brain. Perturbed oxidation of BCAAs and other changes in the brain amino acid metabolism are known to be associated with thiamine deficiency [6, 7]. As discussed in the Introduction, metformin is a commonly used antidiabetic drug, whose well-known but underestimated side effect is inhibition of thiamine transporters. Amprolium is a veterinary drug whose antiparasitic action is based on the blockade of thiamine transport. Although the parasites have a higher sensitivity to the such blockade than their hosts [50], depending on a dose, the hosts can also be affected. So amprolium is used to model thiamine deficiency in animals and animal cells [16, 25]. Because impaired thiamine status is a common comorbidity in diabetes, we use chronic combined administration of metformin and amprolium to model the effect of metformin in diabetic patients who have undiagnosed thiamine insufficiency. High sensitivity of the nervous system to disturbances

in the thiamine status is in particular due to the thiamine dependence of the metabolism of amino acid neurotransmitters or their precursors [30, 35, 41, 51]. Hence, disturbed thiamine status in the brain may also influence behavioral and ECG parameters, thus justifying our interest in the effects of the thiamine transporters inhibitors on the brain metabolism.

We show that chronic administration of metformin/amprolium increases the levels of BCAAs in the brain (Fig. 4), which is in agreement with the results of independent studies on the metformin-impaired BCAA degradation [4]. The disappearance of significant correlations between the contents of these amino acids and activities of ThDP-dependent enzymes (Table 1) in the treated animals links changes in the BCAA levels with alterations in the thiamine-dependent metabolism. The absence of the effect of metformin/amprolium treatment on the content of total brain ThDP (Fig. 2) may be partly due to the buffering role of strong inhibition of thiamine diphosphokinase by ThDP [52]. An increased saturation of TKT and OGDC with the coenzyme by the end of treatment (Fig. 3) suggests compensatory adaptations of ThDP-binding enzymes in response to the chronic administration of thiamine transport inhibitors. Such adaptations are inferred from non-monotonous changes in the physiological parameters during the experiment (Fig. 5) and correlations of these time-dependent parameters with the end-stage levels of ThDP and ThDP-dependent enzymatic activities (Table 2). Apart from the tissue ThDP levels, the saturation of ThDP-dependent enzymes with ThDP may also depend on the allosteric effects [53] or posttranslational modifications of the enzymes [32, 44]. A higher extent of TKT saturation with ThDP has also been observed in various pathological conditions in independent studies (see [44] and references therein). Irrespectively of complex molecular mechanisms involved, the increase in the saturation of brain TKT and OGDC with ThDP after chronic administration of metformin/amprolium indicates that the drugs affect central glucose metabolism in both the cytoplasm and mitochondria. Although the treatment-induced changes in the average levels of redox indicators (Fig. 2) and activities of ThDP-dependent enzymes (Fig. 3) appear minor, the symptoms of oxidative stress (decrease in the content of the antioxidant carnosine and a trend toward increase in the content of oxidized glutathione, Fig. 2) occur along with significant disturbances in the amino acid metabolism and elevation of ammonia (Fig. 4). These changes are accompanied by the treatment-induced modifications of animal behavior and ECG (Fig. 5).

The effects of chronic administration of metformin and amprolium on the content of brain amino acids and relationship between their levels (Fig. 4, Table S1 in the Online Resource 1) are strikingly similar to those observed in the brain metabolism after acute hypoxia [54]. Both insults increase the levels of free amino acids in the brain and strengthen their correlations to each other. The similarity of effects of these very different metabolic perturbations on the brain metabolism of amino acids implies strong impairment of oxidative energy metabolism not only in the case of acute hypoxia, but also after chronic administration of metformin and amprolium. As thiamine is essential for oxidative metabolism, the similarity supports the thiamine dependence of the perturbed metabolism of amino acids after chronic administration of metformin and amprolium inhibiting the intracellular thiamine transport.

The elevated levels of free amino acids and ammonia, with the strongest increase in the content of free methyllysine (Fig. 4), a product of proteolytic degradation, point to perturbations in proteostasis at the end of the treatment. A well-known mediator of the metformin action, AMPK induces autophagy under nutrient stress [55-57]. The decrease in the intracellular thiamine levels at the early stages of treatment may represent a stress signal that activates autophagy by the end of the treatment.

Hyperammonemia is often associated with perturbed amino acid metabolism, aberrant proteostasis, and oxidative stress [58, 59]. Dysregulation of ThDP-dependent enzymes induces hyperammonemia by causing insufficiency of the urea cycle due to the deficit of acetyl-CoA and ATP [60]. The involvement of ThDP-dependent metabolism in brain hyperammonemia caused by chronic administration of metformin/amprolium, is supported by a strong correlation between the brain levels of ThDP and NH_3 at the end of the treatment, which is not observed in the control animals (Table 1). The increase in the OGDC saturation with ThDP as an adaptation to reduced thiamine content may contribute to hyperammonemia by activating amino acid degradation in the TCA cycle, as OGDC catalyzes the rate-limiting step of the cycle. Indeed, not only the levels of ThDP, but also the activities of ThDP-dependent enzymes in the brain strongly change their associations with the levels of amino acids (Table 1).

In view of the significance of α-aminoadipate as a marker of diabetes [3] and degradation of this compound via metabolic pathway mediated by ThDP-dependent 2-oxoadipate dehydrogenase [61], the treatment-induced correlations between the levels of α-aminoadipate and ThDP or activities of ThDP-dependent dehydrogenases (Table 1), are worth noting. The activity of mitochondrial 2-oxoadipate dehydrogenase complex cannot be assessed in brain homogenates because of a much higher expression of 2-oxoglutarate dehydrogenase compared to 2-oxoadipate dehydrogenase and the fact that both complexes can utilize 2-oxoglutarate and 2-oxoadipate as substrates [8, 51, 62].

Remarkably, however, *in vivo* inhibition of 2-oxoadipate dehydrogenase by the active site-directed inhibitors decreases the carnosine content and increases the β-alanine content in the rat brain [51]. The same changes are observed after chronic administration of metformin/amprolium (Figs. 1 and 4), suggesting that the treatment-induced thiamine deficit inhibits 2-oxoadipate dehydrogenase due to the decreased availability of ThDP for the enzyme saturation. This treatment-induced increase in the OGDC affinity to ThDP (Fig. 2) may contribute to the decreased availability of ThDP to 2-oxoadipate dehydrogenase. The inability of 2-oxoadipate dehydrogenase to successfully compete with OGDC for ThDP is supported by existence of a positive correlation between the endogenous level of holoOGDC and the content of α-aminoadipate, an upstream intermediate of the 2-oxoadipate dehydrogenase substrate, in the treated, but not control, animals (Table 1). This correlation means that the higher the activity of OGDC endogenously saturated with ThDP, the higher the brain α-aminoadipate level. In other words, the higher the OGDC activity, the less efficient the degradation of the α-aminoadipate transamination sibling 2-oxoadipate by 2-oxoadipate dehydrogenase. The inhibition of 2-oxoadipate dehydrogenase by the chronic administration of metformin/amprolium is further supported by the positive correlation between the levels of NAD^+ and tryptophan and negative correlation between the NAD^+ content and α-aminoadipate content in the treated, but not in control, rats (Figs. 6 and 7). As noted elsewhere [62], inhibition of 2-oxoadipate dehydrogenase may promote NAD^+ biosynthesis from tryptophan through quinolinic acid, a pathway alternative to the tryptophan degradation through α-aminoadipate. Therefore, correlation analysis provides evidence that chronic administration of metformin/amprolium inhibits 2-oxoadipate dehydrogenase, thus increasing the impact of tryptophan-dependent NAD^+ biosynthesis in the rat brain. Induction of metabolic markers of 2-oxoadipate dehydrogenase dysfunction and α-aminoadipate accumulation in the brain deserves special attention in view of the known association between the blood levels of α-aminoadipate or dysfunction of 2-oxoadipate dehydrogenase and diabetes, obesity, and cardiovascular disorders (reviewed in [51]).

The metformin/amprolium-induced changes in the rat brain biochemistry are associated with changes in the rat behavior and ECG parameters. Administration of metformin/amprolium perturbs the balance between the sympathetic and parasympathetic regulation, which is evident from the correlations between the ECG parameters and freezing time.

It is known that amprolium reduces the exploratory activity [23], while metformin demonstrates anxiolytic and antidepressive effects [63, 64]. In our study, the combined administration of metformin and amprolium produces a borderline anxiolytic action ($p = 0.05$ for the treatment factor, Fig. 5) accompanied by an increase in the locomotor activity, which presumably contributes to increases in the number of entries to the central zone and cumulative index of exploratory activity in the treated vs. control animals. These effects are linked to the time-dependent increases in the number of entries to the central zone and the heart rate, observed in the treated animals only (Fig. 5). The absence of these changes in the control rats may manifest their better accommodation to repeated testing, compared to the treated rats.

Along with the inhibition of thiamine transport, other mechanisms of the metformin action, in particular its activation of the AMPK-dependent pathways, may underlie the complexity of biochemical and physiological effects of the combined administration of metformin and amprolium. Our study highlights a conditional nature of metformin action. The benefits of metformin administration can be reduced by exacerbation of thiamine insufficiency due to various comorbidity factors, including nutritional problems and/or genetic variants of thiamine transporters in humans.

CONCLUSION

Our scheme of chronic administration of thiamine transport inhibitors metformin and amprolium does not significantly change the final levels of ThDP and total activities of ThDP-dependent enzymes in the treated vs. control rats. However, the non-monotonous changes in the behavior and ECG of the treated rats in the course of experiment, and the relationships of behavioral or ECG parameters with the levels of ThDP or activities of ThDP-dependent enzymes at the end of experiment suggest adaptation to the impaired thiamine availability. The treatment with metformin/amprolium switches the metabolic effects of ThDP and ThDP-dependent enzymes from the antioxidant and nitrogen-sparing to the pro-oxidant and hyperammonemic ones. This metabolic switch is accompanied by an increase in the heart rate in the treated, but not control, animals, and locomotion-related elevation of the exploratory activity in the treated vs. control rats.

Supplementary information. The online version contains supplementary material available at https://doi.org/10.1134/S0006297924100043.

Contributions. A.V.G. planned and performed animal experiments; A.V.A. assayed dehydrogenases of 2-oxo acids; O.N.S. assayed TKT and ThDP; A.L.K. quantified amino acids; A.V.G., A.V.A., and V.I.B. analyzed and visualized results. V.I.B. developed the study concept and design, supervised the study, and wrote the manuscript.

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Ethics declarations. This work does not contain any studies involving human subjects. All animal experiments were approved by the Bioethics Committee of the Lomonosov Moscow State University (protocol 137-d 11.11.2022). The authors of this work declare that they have no conflicts of interest.

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