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EXPERIMENTAL ARTICLES

Biogenic Monoamines, Their Precursors, and Metabolites in the Brain of Rats under Experimental Circulatory Failure

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Abstract—The aim of the study was to establish the effect of experimental chronic circulatory failure on the levels of biogenic monoamines, their precursors, and their metabolites in rat brain structures. Thirteen weeks after experimental narrowing of the abdominal aorta to 1 or 0.7 mm, the levels of biogenic monoamines, their precursors, metabolites, kynurenine, and kynurenic acid were measured by reverse phase HPLC in rat brain structures. It was found that circulatory failure is accompanied by a decrease in the synthesis and functional activity of a mediator in the serotonergic system, which was evaluated using the level of the final metabolite 5-hydroxyindoleacetic acid. In the cerebral hemispheres, this may be partially related to deficiency of the precursor. The activation of kynurenic acid synthesis in the cerebral hemispheres and the brain stem is also likely. Changes in the indices that characterize central catecholaminergic systems were less pronounced.

Keywords: heart failure, brain, tryptophan, catecholamines, serotonin

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INTRODUCTION

Among the biochemical changes detected in patients with cardiovascular diseases, the frequency of metabolic disorders of free amino acids, including those related to the synthesis of biologically active compounds (hormones, mediators) involved in the regulation of rhythm, conduction, and contractile function, is relatively high [1, 2]. Among those compounds, an important role belongs to aromatic amino acids, mainly tryptophan and the products of its transformations via both the main metabolic pathway, hydroxylase-mediated pathway, which leads to the formation of serotonin and melatonin; and the 2,3-dioxygenase-mediated pathway, whose products are kynurenine and anthranilic acid and their derivatives [3]. It was shown that these derivatives influence the energy production reactions [4]. Serotonergic mechanisms of regulation of cardiac function mediated by 5-HT_2 receptors include the expression of these receptors [5]. It was shown that degradation of biogenic amines contributes to the production of prooxidants in the heart [6]. On the other hand, the high prevalence of depressive disorders in coronary heart disease worsens the prognosis and requires special approaches to therapy [7]. There are approaches related to the metabolic correction of ischemic lesions in coronary heart disease and chronic heart failure (CHF), including modulation of serotonergic regulatory mechanisms [8], and modulation of kynurenine metabolism [9] in concomitant depressive disorders.

It is known that tryptophan deficiency reduces heart rate variability [10] and affects the course of depressive states. Depressive states increase the risk of sudden cardiac death or progression of heart failure [11], including depression associated with metabolic disorders of tryptophan along the pyrrolase (kynurenine) pathway [12].

The purpose of this study was to establish the effect of chronic circulatory failure on indices characterizing the main monoaminergic systems of the rat brain, including levels of amino acid precursors and metabolites, as well as the main indicators of the pyrrolase pathway of tryptophan cleavage, the levels of kynurenine and kynurenic acid.

MATERIALS AND METHODS

Chronic circulatory failure (CF) was reproduced in rats by artificially narrowing the lumen of the abdominal aorta above the origin of the renal arteries by application of a metal spiral restricting the aortic lumen [13, 14]. Forty male albino rats of a heterogeneous stock were used in the experiment; body weight at the beginning of the experiment was 140–200 g. Animals had a standard vivarium diet with free access to water and adapted before surgery for at least 14 days. Before the experiment, rats were randomized by body weight. Operations were performed under ketamine anesthesia (100 mg/kg, intramuscularly). A part of the

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abdominal aorta was isolated above the origin of renal artery. Next, the parietal peritoneum was dissected, 2.5–3 turns of a spiral made of 0.27 mm thick nichrome wire was applied to a 5 mm long section of the abdominal aorta, which limited the lumen of the abdominal aorta to 1 or 0.7 mm.

During the first two days after the operation, the animals were treated with a Darrow solution (glucose 50.0, potassium chloride 2.7 g, sodium chloride 9.0 g, water 1 L), then transferred to normal maintenance and enteral nutrition.

Animals of one of the groups were subjected to all stages of the operation, except for the imposition of a spiral (sham operated control).

After the operation, the animals were kept on a standard vivarium diet with free access to water for 13 weeks.

This model reproduces isometric hyperfunction of the heart, causing severe hypertrophy of the heart muscle. According to hemodynamic parameters [13, 14], circulatory failure corresponds to failure observed during arterial hypertension and aortic stenosis. Since the development of compensatory hyperfunction of the heart, hypertrophy, and the clinical signs of circulatory failure, have a phasic nature, we chose the most stable phase of long-term compensation, which is observed in animals at 1–2 months. During this time, as a result of rat growth, the degree of narrowing of the lumen of the abdominal aorta progresses, reaching a 2.5–3-fold narrowing which subsequently remained relatively stable [13].

Twelve hours before decapitation, animals were deprived of food, but free access to water was maintained. The experiment was performed in accordance with the rules and standards of bioethical treatment of experimental animals and approved by the committee on biomedical ethics of Grodno State Medical University.

After decapitation, the brain was quickly removed in a cold environment, and the regions of interest (cerebral hemispheres, striatum, midbrain, and hypothalamus) were isolated [15] and frozen in liquid nitrogen.

Tissue samples (20–80 mg) were weighed without thawing, and treated with a 10-fold volume of 0.2 M perchloric acid solution containing 40 mg/L EDTA, 40 mg/L $Na_2S_2O_5$, and 1 μM vanillic acid (internal standard), and immediately homogenized at 400–600 rpm in 1.6 mL microtubes with a specially fitted Teflon pestle. The samples (perchloric acid extracts) were then centrifuged for 15 min at 4°C and 16000 *g*. Supernatants were immediately removed by aspiration and stored until study at −18°C. The standard solutions used to calibrate the chromatographic system were treated in a similar manner.

Measurement of tyrosine, tryptophan, biogenic amines, their precursors, and metabolites is based on ion-pair HPLC of tissue perchloric extracts with detection by natural fluorescence in our modifications [16]. Separation conditions were as follows: Zorbax Plus C_{18} column (Agilent Technologies) 2.1 × 150 mm thermostatted (or maintained) at 27°C. Mobile phase: 0.1 M NaH_2PO_4, 0.033 M CH_3COOH, pH 3.45; 110 mg/L sodium octanesulfonate, 50 mg/L EDTA, 5.1% (V/V) acetonitrile. Flow rate was 0.2 mL/min. Fluorescence was excited at 280 nm and measured at 340 nm. The volume of input samples (perchloric acid extracts) was 5–10 μL. Identification of the compounds and quantitative processing of the chromatograms were performed using the internal standard method. The mixture of standards included: 3,4-dihydroxyphenylalanine (DOPA), tyrosine (Tyr), norepinephrine (NE), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), epinephrine (E), 5-hydroxytryptophan (5-HTP), normetanephrine (NM), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), tryptophan (Trp), homovanilinic acid (HVA), 3-methoxy tyramine (3-MT), and serotonin (5-HT) at concentrations of 1 μmol/L.

To measure the key metabolites of the kynurenine pathway of tryptophan metabolism, we used a previously modified reverse-phase HPLC method with isocratic elution and combined detection using absorption and fluorescence [17]. Kynurenine (KYN) was detected by absorption at 362 nm, kynurenic acid (KYNA) was detected by fluorescence at wavelengths of 244/386 nm. Tryptophan, whose peak was visible on the chromatograms, was used as an internal standard, the reference level for which was taken from the chromatogram of biogenic monoamines (see above).

We used a Zorbax SB C_{18} column (3.5 μm, 2.1 × 150 mm), which was thermostatted at 40° C. The mobile phase was as follows: 2.6 g sodium acetate, 60 μL CH_3COOH, 2.2 g of anhydrous zinc acetate per 1 L H_2O, 4% acetonitrile (*V*/*V*). Immediately before injection of samples (8 μL), perchloric acid in the samples was neutralized by the addition of an equal volume of a 1M sodium acetate solution.

For chromatographic measurements, an Agilent 1200 HPLC instrument was used in a configuration with a G1311A 4-channel solvent delivery system with a vacuum degasser, a G1329A thermostatted autosampler (ALS), a G1316A column thermostat, a G1321C fluorescence detector, and a G1315D diode array detector. In the experiments reagents of at least Aldrich chemically pure grade standards and triple distilled water were used to prepare the mobile phases. During sample preparation, a Biofuge Primo R + centrifuge with a cooled rotor was used.

Data aquisition and chromatogram processing were performed using the Agilent ChemStation C.01.05 software with manual correction of the baseline using internal standard quantitation and single-level calibration.

For the studied indices, the basic parameters of descriptive statistics were determined. The normality of distribution was estimated using the Kolmogorov–Smirnov and Shapiro–Wilk criteria. For all indices, the group was considered homogeneous. Indices in the groups were compared using the Student's *t* test for independent samples, taking into account the comparison of variances. When the distribution was significantly different from the normal or there were significant differences in the variances of the values in the groups, the Mann–Whitney median test was used to check the validity of paired comparisons. Groups of animals with a spiral with a diameter of 1 mm and 0.7 mm were considered independent, when comparing the experimental groups with the intact and sham operated controls, one-way analysis of variance (ANOVA) and Tukey's test were used to correct for multiple comparisons.

Correlations between certain variables within groups were determined by constructing Pearson correlation matrices.

To identify the significance of the contribution of individual indices to the complex characteristics of the pool of free amino acids and related compounds in groups, as well as to identify the most informative (affected by experimental treatment) indices, discriminant analysis was used. In this case, a step-by-step procedure was used; the F-criterion for inclusion of variables was 1.0, and 0.0 for exclusion, unless other values are indicated. To evaluate the significance of differences in pools in all compared groups (group discrimination), the Wilks' lambda value was used.

In all statistical analysis, the critical level of significance p was 0.05. The data analysis was performed using the Statistica 10.0 software package (SN AXAR207F394425FA-Q).

RESULTS AND DISCUSSION

At 13 weeks after the operation, the sham operated animals (sham control) did not show a significant increase in heart weight, both in absolute values and in relation to body weight (Table 1). In animals with narrowed lumen of the abdominal aorta to a value of 1 mm, the relative weight of the heart (MS/MT) did not undergo significant changes. In this group, three animals developed abdominal aortic aneurysms, which suggests a mixed mechanism of heart failure (isometric + isotonic hyperfunction), for which the development of severe myocardial hypertrophy is less characteristic. Regardless of the presence of aneurysms, the aortic lumen above and below the narrowing was clearly different. When the superimposed spiral had a diameter of 0.7 mm, 13 weeks after the operation, severe cardiac hypertrophy developed (Table 1, $p < 0.0001$), and the absolute and relative weights of the heart had a normal distribution. None of these animals developed aortic aneurysms, which indicates a more "pure" reproduction of isometric overload of the heart, which is also evidenced by a larger aortic lumen above the superimposed spiral. Thus, the application of a spiral on the abdominal aorta above the origin of the renal arteries, which limits the lumen of the aorta to 0.7 mm, allows one to obtain pronounced myocardial hypertrophy.

In the cerebral hemispheres of rats, there was a decrease in the levels of tyrosine and tryptophan, as well as 5-hydroxytryptophan (Table 1). This was accompanied by a decrease in serotonin levels, and in the group with a 1 mm spiral, also by a decrease in 5-hydroxyindoleacetic acid. This may mean depression of the serotonin system, which is mediated by the availability of the precursor, and possibly also a decrease in the tryptophan hydroxylation rate, since the level of 5-hydroxytryptophan in both experimental groups decreased more than the level of tryptophan (almost twofold).

According to the discriminant analysis of the pool of the studied parameters, the classification of the cases was completely correct between the control and experimental groups, although it was not completely correct between the intact and sham operated control, as well as between the two experimental groups, which indicates the presence of a qualitatively different state of aminergic systems in the presence of CF, but not surgery and anesthesia. The Wilks' lambda value of 0.02214 ($p < 0.0001$) indicates pronounced differences between the ratios of the components in the pool of the studied compounds in groups.

The value of root 1 (Fig. 1a) characterizes the differences between the experiment and control, i.e. CF effects. The indices that correlate most strongly with root 1 are the levels of kynurenic acid and tyrosine (negative relationship with root value) and the levels of tryptophan, 5-hydroxytryptophan, and kynurenine (positive correlation). Since the experimental groups had lower values of root 1, this may indicate the presence of a decrease in the synthesis of a mediator in the serotonin system in CF, as well as the active conversion of kynurenine to kynurenic acid in this part of the brain, since the blood-brain barrier is impermeable for the latter. As for root 2, its value was lower in the sham operated control compared the intact control, and in the group of animals with stronger narrowing of the aortic lumen it was lower compared to the group with a narrowing to 1 mm. This root was also influenced to a greater extent by the level of kynurenic acid (downward) and kynurenine (upward) but the contribution of this root to the differentiation of groups was much smaller. Root 1 describes 83.3% of the variance of the sample, and roots 1 and 2, 97.1% of the variance of the sample. The levels of 5-hydroxytryptophan, kynurenic acid, kynurenine, and tyrosine were the most "informative" in assessing intergroup differences.

It is known that central synthesis of kynurenic acid may underlie cognitive impairment in schizophrenia

Table 1. The content of biogenic monoamines, their precursors, their metabolites, and tryptophan metabolites in the cerebral hemispheres of rats 13 weeks after artificial narrowing of the lumen of the abdominal aorta, nmol/g of tissue. Mh/mb, the ratio of heart weight to body weight (%)

Index	Intact control, $n = 8$	Sham operated control, $n = 8$	Narrowing of the aorta to 1 mm, $n = 7$	Narrowing of the aorta to 0.7 mm, $n = 8$
Mh/mb	3.26 ± 0.14	3.45 ± 0.089	3.52 ± 0.15	4.65 ± 0.16[1, 2]
Tyr	82.28 ± 4.43	64.58 ± 4.57	67.88 ± 3.64[1]	64.06 ± 4.40[1]
DOPA	0.167 ± 0.058	0.192 ± 0.063	0.092 ± 0.021	0.107 ± 0.024
DA	0.346 ± 0.080	0.273 ± 0.038	0.321 ± 0.044	0.404 ± 0.074
DOPAC	1.44 ± 0.13	0.92 ± 0.14	1.06 ± 0.10[1]	1.12 ± 0.12
HVA	1.28 ± 0.16	1.18 ± 0.06	0.72 ± 0.06[1, 2]	0.97 ± 0.11
NE	6.92 ± 0.43	6.80 ± 0.43	9.87 ± 1.90	9.49 ± 2.80
NM	0.180 ± 0.069	0.088 ± 0.012	0.061 ± 0.005[2]	0.069 ± 0.008
MHPG	9.561 ± 1.953	7.929 ± 1.288	0.83 ± 0.16[1, 2]	1.58 ± 0.27[1.2]
5-HTP	0.289 ± 0.016	0.299 ± 0.037	0.095 ± 0.013[1, 2]	0.155 ± 0.012[1, 2]
Trp	48.48 ± 1.30	46.96 ± 3.01	36.37 ± 1.74[1, 2]	43.77 ± 1.53[1]
5-HT	6.645 ± 0.3943	5.916 ± 0.523	3.749 ± 0.498[1, 2]	5.149 ± 0.482[1]
5-HIAA	3.421 ± 0.266	3.772 ± 0.264	2.021 ± 0.191[1, 2]	3.053 ± 0.283
KYN	1.860 ± 0.353	1.782 ± 0.380	1.564 ± 0.263	2.398 ± 0.491
KYNA	0.045 ± 0.0044	0.075 ± 0.033	0.064 ± 0.0139	0.105 ± 0.0314

Here and in Tables 2–4:
The results are presented as mean ± standard error of the mean; [1]significant differences ($p < 0.05$) compared to intact control; [2]significant differences compared to sham operated control ($p < 0.05$).

[18]. Since kynurenic acid is the only endogenous antagonist of NMDA receptors known so far, it can also mediate glutamatergic hypofunction. Glutamatergic neurotransmission has modulating effects on the dopaminergic system, whose dysfunction is associated with schizophrenia. This, as well as the *n*-anticholinergic activity of kynurenic acid in small doses, can explain the psychotic and cognitive disorders with an increase in kynurenic acid level in the central nervous system [19].

In the brainstem of rats, the nature and direction of changes was similar to that for the cerebral hemispheres, however, a significant decrease in tyrosine level was recorded only for the group of animals that had a spiral with a narrowing to 0.7 mm and in tryptophan level, in the animals with a narrowing to 1 mm (Table 2). The level of 5-HTP decreased, however, only in the group with aortic narrowing to 0.7 mm. Although the level of serotonin did not change, the content of 5-HIAA decreased in both experimental groups, which suggests a decrease in serotonergic functions in the brainstem, but the availability of the precursor and the rate of mediator synthesis changed weakly, according to the 5-HTP level.

Despite the absence of significant changes in the catecholamine levels, the levels of their metabolites HVA and MHPG decreased in this part of the brain. For the latter compound, interpretation of this is difficult, since it undergoes active sulfation, and the sample preparation procedure used allows us to determine the free fraction of MHPG, which leaves open the question about the rate of NE degradation. We were not able to detect changes in the level of normetanephrine, therefore, we cannot argue that a decrease in the level of the metabolite of norepinephrine is associated with a decrease in the activity of the corresponding mediator system. However, it may be assumed that a decrease in dopamine transmission activity occurs, given the decrease in homovanillic acid levels.

Discriminant analysis of the pool of the studied compounds showed that there are pronounced differences in the ratios within the pool of the studied compounds in groups (Wilks' lambda value 0.02112, $p < 0.0001$). The root 1 (Fig. 1b) characterizes the differences between experiment and control, i.e. CF effects. The indices that had the strongest correlations with root 1 are tryptophan and MPEG (negative correlation) and DOPA (positive correlation) levels. Since the experimental groups had lower values of root 1, this may indicate the presence of tryptophan deficiency in CF. The root 2 did not differ in the control groups but in the group of animals with narrowing of the aortic lumen to 0.7 mm, it was lower than in the group with narrowing to 1 mm. The value of this root was influenced by the level of tryptophan (downward) and serotonin (upward). Note that there is a correlation of the levels of the last two compounds in the experimental groups ($r = 0.83$ and 0.84; $p < 0.05$ with

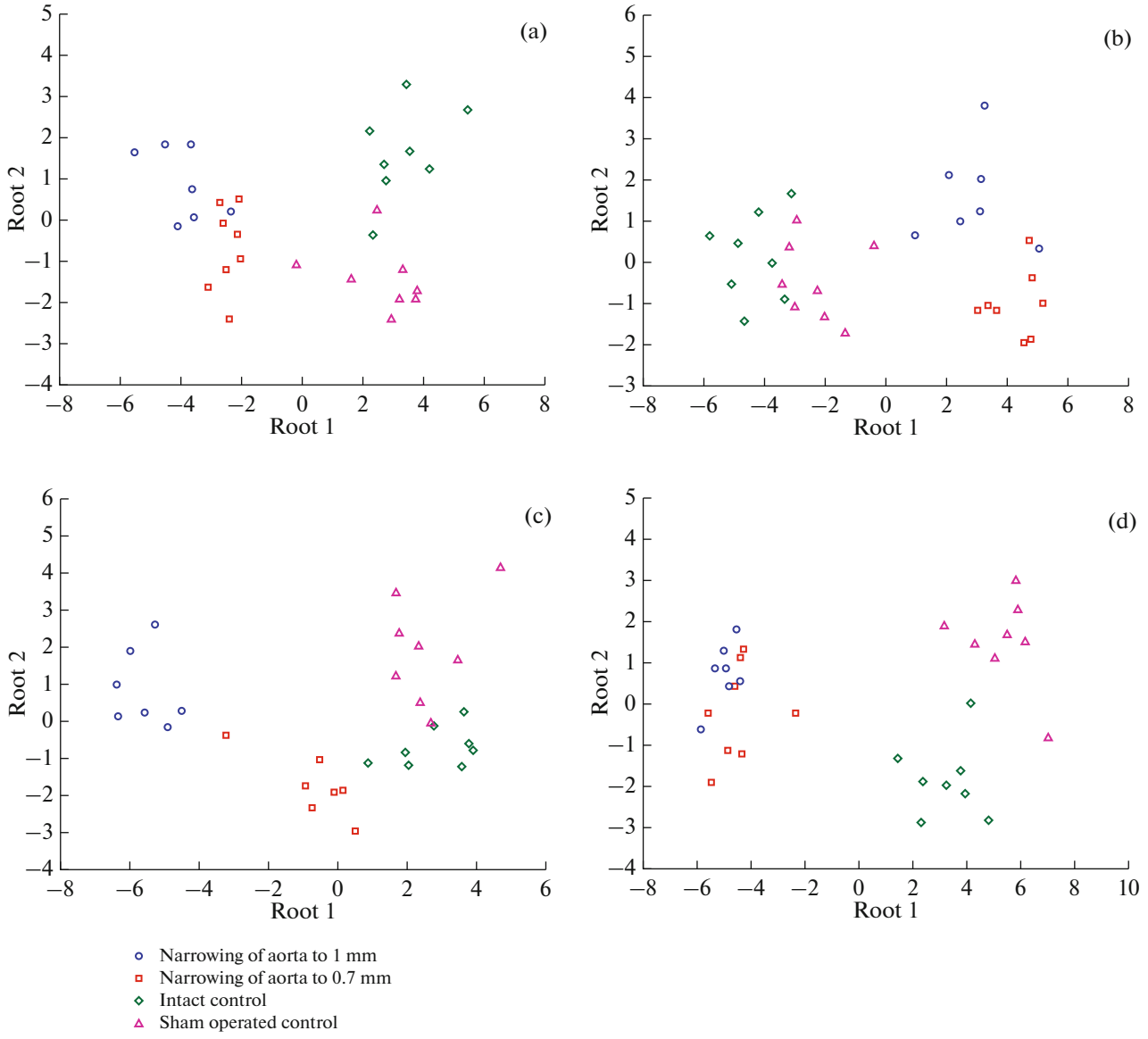

Fig. 1. The location of individual implementations of experimental groups on the plane of the roots of discriminant functions (root 1 and root 2). (a) Cerebral hemispheres; (b) brainstem; (c) hypothalamus; (d) striatum.

narrowing of the aorta to 1 and 0.7 mm, respectively), but not in the sham operated control. Root 1 describes 90.7% of the variance of the sample, and roots 1 and 2, 97.2% of the variance of the sample. The levels of MHPG, tryptophan, and DOPA were the most "informative" in assessing intergroup differences.

In the rat hypothalamus, after narrowing of the the aortic lumen, the levels of tyrosine (in both experimental groups) and tryptophan (only after narrowing to 1 mm) decreased. In both experimental groups, the level of 5-HTP decreased; a decrease in the level of 5-HIAA was observed only after narrowing of the aorta to 0.7 mm (Table 3). Moreover, the level of serotonin in the last group did not differ from the control. As in the brainstem, the level of MPEG decreased significantly in CF, but the level of norepinephrine was also lower than the control, and the decrease in the level of dopamine and its metabolites was less pronounced. Therefore, in this structure of the brain, circulatory failure is associated with a depression of monoaminergic systems, which is mediated by the availability of precursors. Note that more pronounced changes were observed in the group of animals with narrowing of the aortic lumen to 1 mm, in which the development of aortic aneurysms was observed more often, and, therefore, the mechanism of hyperfunction of the heart muscle was more complex.

The last observation illustrates the arrangement of groups on the plane of two roots of discriminant functions (Fig. 1c). The group of animals with narrowing

Table 2. The content of biogenic monoamines, their precursors, their metabolites, and metabolites of tryptophan in the brainstem of rats 13 weeks after artificial narrowing of the lumen of the abdominal aorta, nmol/g of tissue

Index	Intact control, $n = 8$	Sham operated control, $n = 8$	Narrowing of the aorta to 1 mm, $n = 7$	Narrowing of the aorta to 0.7 mm, $n = 8$
Tyr	68.20 ± 3.89	61.78 ± 4.14	61.97 ± 2.21	51.65 ± 4.24[1]
DOPA	0.051 ± 0.006	0.059 ± 0.009	0.082 ± 0.018	0.136 ± 0.017[1, 2]
DA	0.513 ± 0.058	0.507 ± 0.053	0.448 ± 0.039	0.559 ± 0.115
DOPAC	1.027 ± 0.176	1.020 ± 0.127	0.834 ± 0.113	0.901 ± 0.080
HVA	1.165 ± 0.103	1.241 ± 0.132	0.83 ± 0.056[1, 2]	0.846 ± 0.098[1, 2]
NE	7.419 ± 0.923	7.382 ± 0.519	7.751 ± 0.977	6.289 ± 0.945
NM	0.063 ± 0.010	0.109 ± 0.021	0.072 ± 0.016	0.074 ± 0.006
MHPG	17.375 ± 1.520	9.132 ± 2.292[1]	1.07 ± 0.157[1, 2]	0.975 ± 0.150[1, 2]
Trp	43.07 ± 1.40	45.32 ± 1.87	34.76 ± 1.38[1, 2]	37.62 ± 3.18
5-HTP	0.484 ± 0.083	0.464 ± 0.078	0.275 ± 0.073	0.232 ± 0.057[1, 2]
5-HT	8.649 ± 0.486	8.846 ± 0.394	22.402 ± 10.785	7.957 ± 0.983
5-HIAA	8.964 ± 0.394	8.865 ± 0.472	6.41 ± 0.477[1, 2]	5.916 ± 0.794[1, 2]
KYN	1.390 ± 0.158	1.856 ± 0.215	1.312 ± 0.129	1.284 ± 0.220
KYNA	0.030 ± 0.003	0.098 ± 0.036	0.040 ± 0.003	0.044 ± 0.008

Table 3. The content of biogenic monoamines, their precursors, their metabolites, and tryptophan metabolites in rat hypothalamus 12 weeks after artificial narrowing of the lumen of the abdominal aorta, nmol/g of tissue

Index	Intact control, $n = 8$	Sham operated control, $n = 8$	Narrowing of the aorta to 1 mm, $n = 7$	Narrowing of the aorta to 0.7 mm, $n = 8$
Tyr	85.76 ± 3.87	75.81 ± 3.58	56.38 ± 3.51[1, 2]	69.55 ± 2.81[1]
DOPA	0.093 ± 0.013	0.081 ± 0.014	0.086 ± 0.011	0.110 ± 0.006
DA	1.944 ± 0.124	1.741 ± 0.190	0.923 ± 0.343[1]	2.311 ± 0.304
DOPAC	0.933 ± 0.185	1.221 ± 0.167	0.782 ± 0.089[2]	0.917 ± 0.117
HVA	0.953 ± 0.129	1.388 ± 0.132[1]	0.746 ± 0.040[2]	0.916 ± 0.063[2]
NE	45.36 ± 1.83	41.61 ± 1.75	11.37 ± 0.60[1, 2]	38.83 ± 4.54[2]
NM	0.227 ± 0.111	0.071 ± 0.011	0.097 ± 0.023	0.092 ± 0.014
E	0.494 ± 0.051	0.482 ± 0.084	0.161 ± 0.055[1, 2]	0.518 ± 0.070
MHPG	25.48 ± 2.28	22.13 ± 3.401	2.48 ± 0.55[1, 2]	3.84 ± 1.20[1]
Trp	48.50 ± 1.34	50.93 ± 2.70	32.17 ± 2.66[1, 2]	46.43 ± 1.52
5-HTP	1.175 ± 0.064	1.209 ± 0.147	0.406 ± 0.099[1, 2]	0.355 ±0.053[1, 2]
5-HT	11.76 ± 0.38	10.24 ± 0.68	8.76 ± 0.71[1]	10.01 ± 0.91
5-HIAA	7.519 ± 0.240	6.817 ± 0.380	9.205 ± 1.336	5.140 ±0.407[1, 2]
KYN	1.267 ± 0.152	1.601 ± 0.158	1.996 ± 0.360	1.968 ± 0.334
KYNA	0.036 ± 0.002	0.047 ± 0.005	0.049 ± 0.006	0.044 ± 0.004

of the aortic lumen to 1 mm differs more significantly from the control in root 1; the value of root 1 is associated with concentrations of MPEG and 5-HTP (positive correlation) and 5-HIAA (negative correlation). The latter may indicate the presence of a more pronounced depression of serotonergic functions in the group with narrowing of the aortic lumen to 0.7 mm, and inhibition of mediator synthesis in the group with narrowing of the aortic lumen to 1 mm. Both roots describe 89% of the variance of the sample.

In addition to these compounds, the levels of NE, tyrosine, and HVA contributed to the discrimination of groups in this brain structure.

In the rat striatum, CF caused more pronounced shifts in indices related to the serotonin system. So, in both experimental groups, a decrease in the level of tryptophan, 5-HTP (almost threefold), and 5-HIAA was observed, but a decrease in 5-HT was only observed with narrowing of the aortic lumen to 0.7 mm (Table 4). The tyrosine level also decreased in

Table 4. The content of biogenic monoamines, their precursors, their metabolites, and metabolites of tryptophan in the rat striatum 12 weeks after artificial narrowing of the lumen of the abdominal aorta, nmol/g of tissue

Index	Intact control, $n = 8$	Sham operated control, $n = 8$	Narrowing of the aorta to 1 mm, $n = 7$	Narrowing of the aorta to 0.7 mm, $n = 8$
Tyr	84.21 ± 5.58	75.54 ± 4.20	75.69 ± 2.54	61.40 ± 3.03[1, 2]
DOPA	0.121 ± 0.033	0.080 ± 0.014	0.112 ± 0.025	0.167 ± 0.027[2]
DA	73.89 ± 4.65	66.38 ± 2.50	66.96 ± 3.72	61.99 ± 8.78
Dopac	5.647 ± 0.546	4.737 ± 0.187	3.616 ± 0.165[1, 2]	4.018 ± 0.620
3-MT	1.817 ± 0.194	1.731 ± 0.099	2.036 ± 0.156	1.887 ± 0.222
HVA	2.551 ± 0.225	2.781 ± 0.246	2.746 ± 0.320	2.287 ± 0.257
NE	2.512 ± 0.588	2.066 ± 0.209	2.313 ± 0.297	1.725 ± 0.391
MHPG	12.70 ± 1.21	7.74 ± 1.43[1]	2.742 ± 0.876[1, 2]	3.570 ± 0.657[1, 2]
5-HTP	0.299 ± 0.015	0.358 ±0.012[1]	0.109 ± 0.014[1, 2]	0.132 ± 0.011[1, 2]
Trp	51.02 ± 1.54	52.41 ± 2.78	41.02 ± 2.21[1, 2]	43.01 ± 2.36[1, 2]
5-HT	6.191 ± 0.312	6.297 ± 0.221	6.087 ± 0.582	5.058 ± 0.371[1, 2]
5-HIAA	7.045 ± 0.291	7.409 ± 0.330	5.651 ± 0.371[1, 2]	5.082 ± 0.295[1, 2]
KYN	2.419 ± 0.209	2.006 ± 0.240	1.964 ± 0.192	2.139 ± 0.183
KYNA	0.046 ± 0.006	0.040 ± 0.004	0.032 ± 0.001	0.046 ± 0.005

the latter group, but the levels of catecholamines and their metabolites did not change significantly, except for MHPG. Thus, in the striatum, the synthesis and degradation of the mediator in the serotonin system were suppressed due to a decrease in the availability of the precursor.

In this part of the brain, the pool of the studied parameters in both experimental groups underwent the most similar changes (Fig. 1d) compared to the control. The root 1 of the discriminant function, by which the experiment and control groups differed, was most associated with the levels of 5-HTP and 5-HIAA (positive association) and NM (negative association). Root 2 describes only the differences between the intact and sham operated controls. In general, the highest discrimination of groups occurred in the striatum (Wilks' lambda of 0.00792, $p < 0.0001$).

Thus, in all the studied parts of the brain, the effects of CF on the pool of biogenic monoamines, their precursors, and their metabolites were unidirectional. Inhibition of the synthesis of mediators may be associated with a decrease in the availability of mediators (an effect on their transport or peripheral mechanisms of the formation of a pool of free amino acids). Although the absolute values of the concentrations of kynurenine and kynurenic acid did not undergo significant changes, based on the data of discriminant analysis, it may be assumed that the formation of KYNA in the brain increases after CF, at least in the cerebral hemispheres and the brainstem. In the latter, the levels of KYN and KYNA positively correlated in the experimental group ($r = 0.84$, $p < 0.05$ after narrowing of the aortic lumen to 0.7 mm) but not in the control. Although in the cerebral hemispheres of sham operated animals, the levels of KYN and KYNA correlated ($r = 0.84$), the correlation coefficient increased after CF ($r = 0.96$ after narrowing of the aortic lumen to 1 mm). This, along with the discriminant analysis data, speaks in favor of activation of the formation of kynurenic acid in the brain with CF.

CONCLUSIONS

(1) Circulatory failure (CF) caused by narrowing of the lumen of the abdominal aorta is accompanied by unidirectional shifts in the monoaminergic systems in all studied brain structures of rats.

(2) The most permanent manifestation of CF in rats is a decrease in the synthesis and functional activity of a mediator in the serotonergic system, which is evaluated by the level of the final metabolite 5-hydroxyindoleacetic acid. In the striatum and cerebral hemispheres, this may be partially due to tryptophan deficiency.

(3) Experimental CF is not accompanied by pronounced changes in indices that characterize central catecholaminergic systems, except inhibition of the degradation of norepinephrine and, to a lesser extent, dopamine.

(4) The results of the discriminant analysis of the pool of the studied parameters suggest the transamination of kynurenine becomes activated in the cerebral hemispheres and brainstem during CF.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare no conflicts of interest.

Ethical approval. The experiments were performed in accordance with the rules and standards of bioethical treatment of experimental animals (order of the Ministry of Health of the Republic of Belarus no. 274 from April 17, 2006) and approved by the Committee on Biomedical Ethics of the Grodno State Medical University.

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