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FEATURES OF THE STATE OF THE NEUROENDOCRINE-IMMUNE COMPLEX AND ELECTROLYTE-NITROGENOUS EXCHANGE UNDER DIFFERENT VARIATIONS OF URIC ACID METABOLISM IN FEMALE RATS

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Abstract

Background. This article concludes the experimental part of the project "Physiological activity of uric acid". Our group previously identified 4 variants-clusters of uricemia and uricosuria both in rats and in humans. It was shown that the clusters differ from each other in the constellation of immune, autonomic and endocrine parameters as well as plasma and urine electrolytes and nitrogenous parameters, which to one degree or another correlate with uricemia and uricosuria. Because the autonomic, endocrine and immune parameters interact with each other within the neuro-endocrine-immune complex, the purpose of this study is to find out exactly which parameters of the neuro-endocrine-immune complex reflect the specificity of quantitative and qualitative clusters of uric acid metabolism. **Materials and Methods**. Experiment was performed on 60 healthy female Wistar rats 220-300 g. The plasma and urine levels of the uric acid, urea, creatinine, calcium, phosphates, chloride, magnesium, sodium and potassium (also in erythrocytes) as well as glucose (in plasma only) were determined. The parameters of the autonomic, endocrine and immune systems were registered. **Results**. 30 parameters of the neuroendocrine-immune complex and metabolism were identified, which collectively reflect the specificity of quantitative and qualitative clusters of uric acid metabolism. In addition to, by definition, uricemia and uricosuria, they represent markers of vagal tone, sympatho-vagal balance, androgenic, mineralocorticoid and glucocorticoid functions of the adrenal cortex as well as parathyroid activity; exchange of electrolytes (excretion with urine of sodium, calcium and phosphates, plasma levels of sodium, magnesium, chloride, phosphates and potassium and the latter in erythrocytes) and nitrogenous metabolites (creatininuria and plasma urea); as well as immunity parameters (the percentage of lymphoblasts, plasma cells and macrophages in the thymocytogram, the mass of the spleen, the entropy of the splenocytogram and the percentage of macrophages and microphages in it, the percentage of Th-, B- and 0-lymphocytes in the blood immunocytogram as well as blood level of leukocytes in general). **Conclusion**. The quantitative and qualitative clusters of uric acid metabolism are accompanied by specific constellations of parameters of the neuro-endocrine-immune complex and the electrolytes and nitrogenous compounds.

Keywords: uricemia, uricosuria, neuro-endocrine-immune complex, electrolytes, nitrogenous compounds, relationships, healthy female rats.

INTRODUCTION

This article concludes the experimental part of the project "Physiological activity of uric acid" [26]. We previously identified 4 variants-clusters of uricemia and uricosuria both in rats [10] and in humans [21]. Using the method of discriminant analysis, it was shown that the clusters differ from each other in the constellation of immune [11-14], autonomic and endocrine [25] parameters as well as plasma and urine electrolytes and nitrogenous parameters [5], which to one degree or another correlate with uricemia and uricosuria. It is known that autonomic, endocrine and immune parameters interact with each other within the neuro-endocrine-immune complex (NEIC) [4,20,24,27,28]. The purpose of this study is to find out exactly which parameters of the NEIC reflect the specificity of quantitative and qualitative clusters of uric acid metabolism.

MATERIAL AND METHODS

Experiment was performed on 60 healthy female Wistar rats 220-300 g. Of these, 10 remained intact, while others received drinking water of various compositions during the week. The day after the completion of the drinking course in all rats assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variation scope (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [2]. Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible as well as taking the thymus, spleen and adrenal glands.

The plasma and urine levels of the uric acid (uricase method), creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), glucose (in plasma only; glucose-oxidase method), calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), magnesium (by reaction with colgamite), chloride (mercury-rhodanidine method), sodium and potassium (both also in erythrocytes; by flamming photometry) were determined. The analyzes were carried out according to the instructions described in the manual [9].

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients $(Cap/Pp)^{0,5}$ and $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficients $(1/Cap•Pp)^{0,5}$ and $(Cau•Pu/Cap•Pp)^{0,25}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Nap•Ku/Kp•Nau)^{0,25}$, based on their classical effects and recommendations [27].

In additional, the plasma levels of the hormones of adaptation such as corticosterone, triiodothyronine and testosterone (by the ELISA) as well as daily excretion of 17-ketosteroides (by reaction with meta-dinitrobenzene) were determined.

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [4].

In the blood, the parameters of immunity were determined as described in the manual [23]: the percentage of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant (T-helper) and theophylline-sensitive (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes (by the test of complementary rosette formation with erythrocytes of sheep). Natural killers were identified as large granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytic index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [4,20,28].

The spleen and thymus were removed, weighed and made smears-imprints for counting splenocytogram and thymocytogram [3,4]. For them, as well as leukocytogram, Shannon's entropy was calculated [24,27].

The analyzers “Tecan” (Oesterreich), “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer “СФ-47”.

Digital material statistically processed on a computer using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

As a result of the discriminant analysis [19], 30 recognition parameters were included in the model (Tables 1 and 2). In addition to, by definition, uricemia and uricosuria, they represent **neuro-endocrine** (6) and **immune** (11) systems as well as **electrolytes** (9) and **nitrogenous** metabolites (2) of blood and urine.

The identifying information contained in the 30 discriminant variables is condensed into three roots. The first root contains 56,0% of the discriminant power ($r^*=0,963$; Wilks' $\Lambda=0,002$; $\chi^2_{(90)}=260$; $p<10^{-6}$), second - 25,6% ($r^*=0,924$; Wilks' $\Lambda=0,028$; $\chi^2_{(58)}=150$; $p<10^{-3}$), third - 18,4% ($r^*=0,899$; Wilks' $\Lambda=0,192$; $\chi^2_{(28)}=69$; $p<10^{-4}$).

Calculation of the values of the discriminant roots for each animal as the sum of the products of non-standardized coefficients on the individual values of the discriminant variables together with a constant (Table 3) make it possible to visualize each rat in the information space of the roots (Fig. 1).

Table 1. Summary of the step-by-step analysis of NEIC and metabolism parameters, ranked according to the Λ criterion

Variable enter	F to enter	p-value	Lambda	F-value	p-value
Uricemia	45,6	10^-6	0,290	45,6	0,000000
Parathyroid Activity	10,2	10^-4	0,187	24,1	0,000000
Magnesium Plasma	5,61	0,002	0,142	18,0	0,000000
Macrophages Thymus	4,05	0,012	0,116	14,7	0,000000
Creatinine Excretion	4,62	0,006	0,091	13,2	0,000000
MxDMn as Vagal tone	3,43	0,024	0,076	12,0	0,000000
0 Lymphocytes Blood	3,31	0,027	0,063	11,1	0,000000
Phosphates Excretion	2,80	0,050	0,054	10,3	0,000000
Macrophages Spleen	2,50	0,071	0,047	9,7	0,000000
T helper Lymphocytes	2,71	0,056	0,040	9,2	0,000000
Urea Plasma	2,32	0,088	0,035	8,8	0,000000
Potassium Erythrocytes	1,86	0,149	0,031	8,3	0,000000
Phosphates Plasma	1,93	0,139	0,027	8,0	0,000000
Potassium Plasma	3,01	0,041	0,023	7,9	0,000000
B Lymphocytes Blood	1,80	0,163	0,020	7,6	0,000000
Entropy Splenocytogram	1,63	0,198	0,018	7,3	0,000000
17-Ketosteroides Excretion	1,80	0,162	0,016	7,1	0,000000
Corticosterone	1,53	0,222	0,014	6,9	0,000000
(Ca/K)^0,5 Plasma	1,49	0,232	0,013	6,7	0,000000
Calcium Excretion	1,92	0,144	0,011	6,6	0,000000
Leukocytes Blood	1,41	0,255	0,010	6,4	0,000000
Plasmocytes Thymus	1,91	0,146	0,008	6,3	0,000000
Microphages Spleen	1,70	0,185	0,007	6,2	0,000000
Spleen Mass Index	1,43	0,251	0,006	6,1	0,000000
Mineralocorticoid Activity	1,75	0,177	0,006	6,0	0,000000
Sodium Excretion	4,10	0,015	0,004	6,4	0,000000
Lymphoblastes Thymus	2,04	0,129	0,003	6,4	0,000000
Uricosuria	1,89	0,153	0,003	6,4	0,000000
Chloride Plasma	1,27	0,305	0,002	6,3	0,000000
Sodium Plasma	1,62	0,208	0,002	6,3	0,000000

Table 2. Summary of the analysis of discriminant functions for NEIC and metabolism parameters, ranked by structural coefficient

Step 30, N of vars in model: 30; Grouping: 4 grps; Wilks' Λ: 0,0021; approx. $F_{(91)}=6,3$; $p<10^{-6}$

	Clusters of Uric acid Exchange (n)				Parameters of Wilks' Statistics					
Variables currently in the model	S-Un- (15)	Sn+U± (19)	SnUn+ (17)	S+Un+ (9)	Wil-ks' Λ	Parti-al Λ	F-re-move	p-level	Tole-rancy	Norm (10)
Uricemia, µM/L	**259**	865	554	**1379**	0,007	0,290	22,0	10^{-6}	0,380	662
Uricosuria, µM/100g•d	**3,31**	6,63	5,46	**7,00**	0,002	0,838	1,74	0,182	0,236	5,72
MxDMn HRV, msec	**26**	39	45	**101**	0,003	0,753	2,96	0,050	0,219	53
0- Lymphocytes, %	**15,5**	22,8	21,8	**23,1**	0,002	0,748	3,03	0,047	0,430	22,2
K Erythrocyt, mM/L	**85,0**	87,3	86,2	**89,6**	0,002	0,826	1,89	0,155	0,439	87,0
Na Excr, µM/100 g•d	**118**	184	149	**201**	0,003	0,620	5,52	0,004	0,104	135
Urea Plasma, mM/L	**9,39**	8,76	9,29	**5,29**	0,003	0,690	4,04	0,017	0,255	7,42
Plasmocytes Thym, %	**2,40**	1,83	1,88	**1,67**	0,002	0,748	3,03	0,046	0,414	1,80
Macrophages Thy, %	3,07	**3,56**	2,88	2,11	0,003	0,714	3,61	0,026	0,347	2,70
Macrophags Spleen,%	7,87	**9,11**	8,18	7,89	0,002	0,925	0,73	0,544	0,204	7,90
Phosph E, µM/100 g•d	9,3	**12,7**	10,4	9,0	0,003	0,720	3,49	0,029	0,061	9,4
17-KS, nM/100g•24h	55	**83**	69	62	0,002	0,886	1,16	0,344	0,067	61
Creatin E, µM/100g•d	8,6	**12,6**	12,6	11,9	0,002	0,863	1,43	0,257	0,115	8,7
T helper Lymphoc, %	30,5	**32,0**	31,3	28,4	0,003	0,665	4,54	0,010	0,419	31,5
Entropy Splenocytogr	0,752	**0,761**	0,751	0,741	0,003	0,716	3,57	0,027	0,389	0,753
Microphag Spleen, %	13,8	**11,8**	13,1	13,9	0,003	0,635	5,16	0,006	0,352	13,0
B Lymphocytes, %	16,3	**15,1**	16,4	16,3	0,003	0,592	6,20	0,002	0,324	16,0
Mineralocort Activity	3,12	**2,83**	3,09	3,03	0,004	0,490	9,36	10^{-4}	0,081	2,73
Mg Plasma, mM/L	0,45	0,78	**1,27**	0,84	0,003	0,677	4,29	0,013	0,360	0,88
Phosphat Plas, mM/L	0,46	1,11	**1,19**	0,84	0,002	0,827	1,89	0,155	0,105	0,72
Corticosterone, nM/L	437	422	**532**	321	0,003	0,677	4,28	0,013	0,225	482
Spleen MI, µg/g BM	264	287	**321**	304	0,003	0,793	2,35	0,095	0,403	312
Ca Excr, µM/100 g•d	2,89	4,17	**4,27**	2,60	0,002	0,864	1,42	0,259	0,151	2,90
K Plasma, mM/L	3,25	3,37	**3,87**	3,63	0,002	0,834	1,79	0,172	0,208	4,23
Na Plasma, mM/L	127,4	128,7	**130,8**	129,4	0,002	0,848	1,63	0,208	0,042	128,6
Cl Plasma, mM/L	90,7	92,0	**94,8**	93,8	0,003	0,802	2,22	0,109	0,036	94,3
Lymphoblasts Thy, %	7,27	7,06	**7,41**	6,67	0,002	0,861	1,45	0,251	0,443	7,40
Leukocytes Blood,G/L	11,35	11,40	**11,98**	11,56	0,002	0,843	1,68	0,195	0,425	12,68
Parathyroid Activity	2,15	1,71	**1,55**	1,93	0,002	0,866	1,39	0,267	0,077	2,08
$(Cap/Kp)^{0,5}$ as S/V bal	0,92	0,85	**0,79**	0,91	0,002	0,872	1,32	0,289	0,125	0,89

Table 3. Standardized and raw coefficients and constants for NEIC and metabolism parameters included in the discriminant model

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Uricemia	1,303	0,429	-0,408	0,0054	0,0018	-0,0017
Parathyroid Activity	-0,690	-0,101	1,262	-2,055	-0,299	3,756
Magnesium Plasma	0,080	0,415	0,959	0,184	0,950	2,195
Macrophages Thymus	-0,716	0,271	-0,597	-0,679	0,256	-0,566
MxDMn as Vagal tone	0,796	-0,778	0,171	0,0201	-0,0197	0,0043
0 Lymphocytes Blood	0,648	0,434	0,208	0,094	0,063	0,030
Phosphates Excretion	0,335	2,273	-0,243	0,064	0,434	-0,047
Macrophages Spleen	-0,212	0,456	-0,429	-0,121	0,261	-0,245
T helper Lymphocytes	-0,505	0,755	0,310	-0,140	0,209	0,086
Urea Plasma	-0,785	0,622	0,626	-0,279	0,221	0,223
Potassium Erythrocytes	-0,053	0,363	-0,589	-0,0085	0,0580	-0,0941
Phosphates Plasma	-0,141	0,084	1,422	-0,322	0,193	3,255
Potassium Plasma	0,537	0,057	0,811	0,694	0,074	1,049
B Lymphocytes Blood	0,703	-0,819	0,533	0,223	-0,259	0,169
Entropy Splenocytogram	-0,212	0,495	-0,770	-9,673	22,53	-35,07
17-Ketosteroides Excretion	0,215	-1,362	-0,327	0,0054	-0,0341	-0,0082
Creatinine Excretion	0,452	0,415	1,026	0,097	0,089	0,221
Corticosterone	0,723	-0,968	0,432	0,0044	-0,0058	0,0026
$(Ca/K)^{0,5}$ Plasma	0,111	-0,371	-1,052	0,683	-2,277	-6,449
Calcium Excretion	-0,280	-0,526	0,855	-0,121	-0,227	0,370
Leukocytes Blood	0,512	0,316	0,229	0,1095	0,0676	0,0490
Plasmocytes Thymus	-0,017	-0,834	0,138	-0,022	-1,042	0,172
Microphages Spleen	0,499	-0,880	0,420	0,262	-0,462	0,220
Spleen Mass Index	0,459	-0,551	0,272	0,0071	-0,0085	0,0042
Mineralocorticoid Activity	1,473	-2,235	-0,045	1,534	-2,328	-0,047
Sodium Excretion	1,047	-1,758	-0,069	0,0061	-0,0102	-0,0004
Lymphoblastes Thymus	-0,309	0,442	0,267	-0,315	0,451	0,272
Uricosuria	-0,697	0,436	-0,301	-0,236	0,148	-0,102
Chloride Plasma	0,696	-0,993	-2,268	0,110	-0,157	-0,358
Sodium Plasma	-0,450	0,560	1,991	-0,083	0,103	0,367
		Constants		-3,586	-13,865	-2,813
		Eigenvalues		12,73	5,824	4,194
		Cumulative Proportio		0,560	0,816	1

Table 4 demonstrates that the characteristic features of the **S-Un** cluster are a combination of moderate hypouricemia and lower borderline uricosuria with reduced vagal tone and the level of 0-lymphocytes in the blood, lower borderline potassium level and a normal, but minimal for the sample, level of natriuria, on the other hand, a moderately increased percentage of plasma cells in the thymus and a maximum for a sample elevated level of urea in the plasma. Such a constellation of parameters is visualized by the localization of cluster members in the extreme left zone of the first root axis (Fig. 1).

At the opposite pole of the axis are members of the **S+Un+** cluster, which are characterized by a combination of pronounced hyperuricemia and normal, but maximal for the sample, uricosuria with moderately increased levels of vagal tone and natriuria and normal, but maximal for the sample, levels of 0-lymphocytes in the blood and potassium in erythrocytes, instead, a moderately reduced level of urea in the plasma and a normal, but minimal for sampling content of plasma cells in the thymus.

Members of the other two clusters occupy an intermediate position along the axis of the first root and are mixed. Their separation occurs along the axis of the second root. The top position is occupied by the rats of the **Sn+U-+** cluster, in which the upper limit level of uricemia is combined with the maximum for the sample upper limit levels of macrophages in the thymus and spleen as well as urinary excretion of phosphates, creatinine and 17-ketosteroids while normal levels of T-helpers and splenocytogram entropy. Instead, they have a reduced level of macrophages in the spleen, and the level of B-lymphocytes in the blood and mineralocorticoid activity are normal, but minimal for the sample, while in the members of the **SnUn+** cluster, the lower borderline level of uricemia is accompanied to a greater or lesser extent by lower/higher levels of the listed parameters.

Additional delimitation of these clusters occurs along the axis of the third root (Fig. 2). The top position is occupied by rats of the **SnUn+** cluster, which reflects their upper limit plasma levels of magnesium, phosphates, corticosterone and calcium excretion as well as normal, but maximum for the sample, levels of chloride in the plasma, leukocytes in the blood, lymphoblastes in the thymus, and mass index of the spleen in combination with the maximally reduced electrolyte markers of parathyroid activity and sympatho-vagal balance, while the rats placed below the **Sn+U-+** cluster are characterized to a greater or lesser extent by lower/higher levels of the listed parameters.

Table 4. Structural coefficients of NEIC and metabolism parameters, their average Z-values and centroids of discriminant roots for clusters

	Correlations Variables-Roots			S-Un-	Sn+U-+	SnUn+	S+Un+
Root 1(56,0%)	Root 1	Root 2	Root 3	**-4,30**	**-0,43**	**+0,63**	**6,88**
Uricemia	**,393**	,191	-,250	**-1,18**	+0,59	-0,32	**+2,10**
Uricosuria	**,106**	,126	-,010	**-0,45**	+0,17	-0,05	**+0,24**
MxDMn as Vagal tone	**,168**	-,057	-,055	**-0,63**	-0,34	-0,18	**+1,18**
0 Lymphocytes Blood	**,097**	,122	,044	**-1,08**	-0,10	-0,07	**+0,15**
Potassium Erythrocytes	**,062**	,024	-,040	**-0,29**	+0,04	-0,12	**+0,38**
Sodium Excretion	**,040**	,041	-,023	**-0,19**	+0,59	+0,17	**+0,79**
Urea Plasma	**-,125**	,039	,116	**+1,15**	+0,78	+1,09	**-1,24**
Plasmocytes Thymus	**-,081**	-,067	-,027	**+0,76**	+0,04	+0,10	**-0,17**
Root 2(25,6%)	Root 1	Root 2	Root 3	-2,40	**+3,25**	-0,46	-1,99
Macrophages Thymus	-,086	**,137**	-,003	+0,27	**+0,64**	+0,16	-0,44
Macrophages Spleen	-,004	**,129**	-,012	-0,02	**+0,76**	+0,17	-0,01
Phosphates Excretion	-,009	**,120**	,006	-0,02	**+0,52**	+0,16	-0,06
17-Ketosteroides Excretion	,013	**,114**	,014	-0,11	**+0,40**	+0,14	+0,02
Creatinine Excretion	,065	**,104**	,076	-0,03	**+0,90**	+0,88	+0,74
T helper Lymphocytes	-,052	**,101**	,062	-0,31	**+0,14**	-0,07	-0,99
Entropy Splenocytogram	-,051	**,100**	-,002	-0,03	**+0,25**	-0,08	-0,43
Microphages Spleen	,010	**-,188**	-,010	+0,56	**-0,82**	+0,08	+0,63
B Lymphocytes Blood	,007	**-,075**	,032	+0,09	**-0,32**	+0,12	+0,11
Mineralocorticoid Activity	-,005	**-,053**	,022	+0,50	**+0,12**	+0,46	+0,39
Root 3(18,4%)	Root 1	Root 2	Root 3	-1,23	-0,89	**+3,10**	-1,93
Magnesium Plasma	,098	,050	**,300**	-0,72	-0,17	**+0,64**	-0,07
Phosphates Plasma	,086	,196	**,203**	-0,57	+0,85	**+1,02**	+0,28
Corticosterone	-,051	,008	**,187**	-0,36	-0,47	**+0,40**	-1,28
Spleen Mass Index	,062	,012	**,126**	-0,48	-0,25	**+0,09**	-0,08
Calcium Excretion	-,008	,097	**,103**	-0,01	+0,83	**+0,90**	-0,20
Potassium Plasma	,048	,076	**,099**	-1,39	-0,69	**-0,51**	-0,85
Sodium Plasma	,039	,010	**,096**	-0,23	+0,02	**+0,43**	+0,16
Chloride Plasma	,049	-,004	**,095**	-0,50	-0,33	**+0,07**	-0,08
Lymphoblastes Thymus	-,048	-,010	**,094**	-0,16	-0,41	**+0,01**	-0,87
Leukocytes Blood	,006	-,004	**,026**	-0,22	-0,21	**-0,12**	-0,19
Parathyroid Activity	-,068	-,166	**-,260**	+0,13	-0,75	**-1,08**	-0,31
$(Ca/K)^{0,5}$ Plasma	-,012	-,068	-,144	+0,17	-0,27	**-0,58**	+0,11

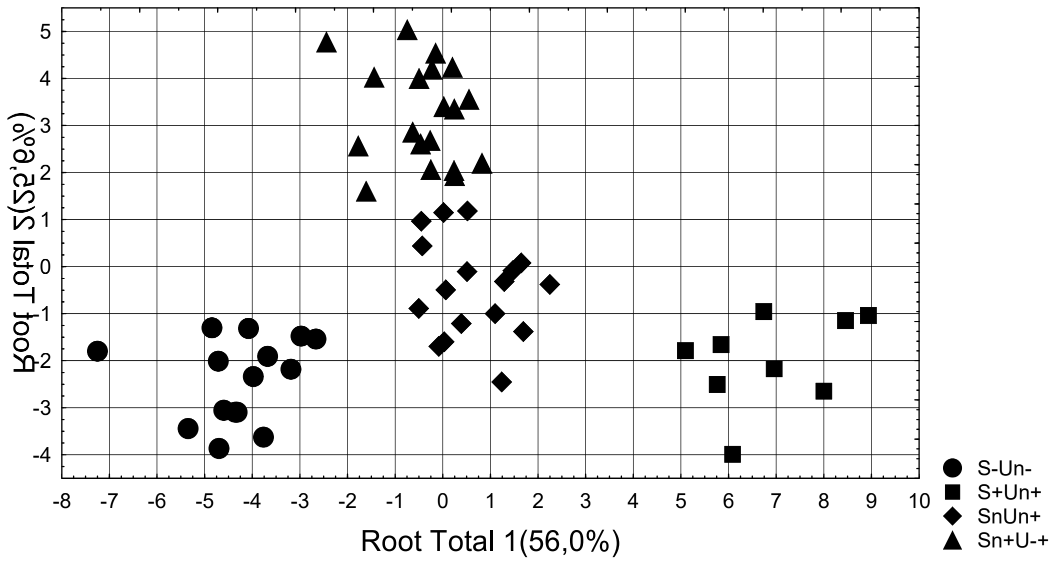

Fig. 1. Scattering of individual values of the first and second discriminant roots of rats of different clusters

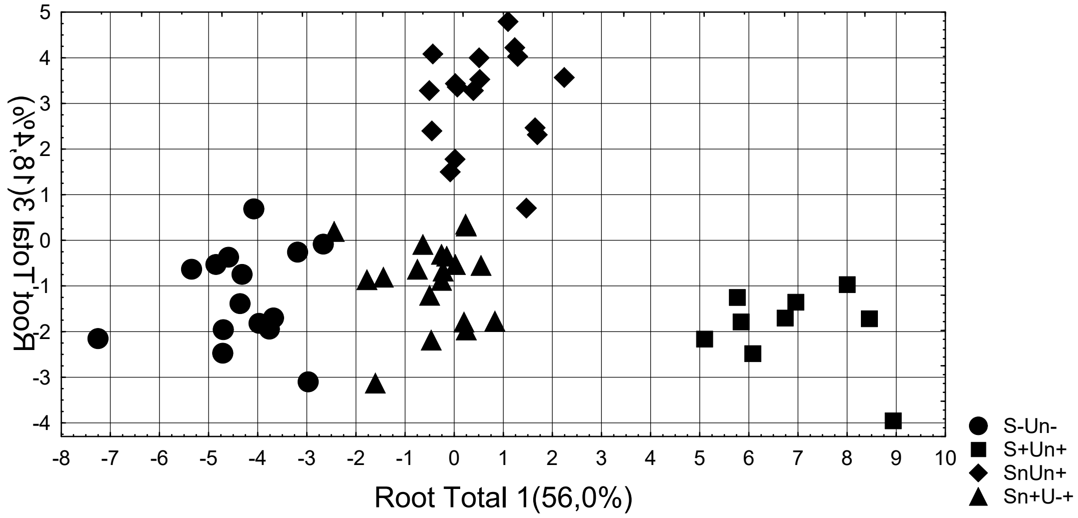

Fig. 2. Scattering of individual values of the first and third discriminant roots of rats of different clusters

The apparent clarity of the demarcation of the four clusters in the information space of the three canonical discriminant roots is documented by the calculation of the Mahalanobis distances between the clusters (Table 5).

Table 5. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=30,3) and p-levels (below the diagonal)

Clusters	S-Un-	S+Un+	SnUn+	Sn+U-+
S-Un-	0	126	47	47
S+Un+	11,4 10^{-6}	0	67	82
SnUn+	6,0 10^{-5}	6,3 10^{-5}	0	31
Sn+U-+	6,3 10^{-5}	8,0 10^{-6}	4,4 10^{-4}	0

The use of classification functions (Table 6) enables error-free retrospective identification of all members of the four clusters (Table 7).

Table 6. Coefficients and constants of classification functions for neuroendocrine-immune and metabolic support of uric acid metabolism clusters

Variable	S-Un- p=,250	S+Un+ p=,150	SnUn+ p=,283	Sn+U-+ p=,317
Uricemia	0,051	0,114	0,074	0,082
Parathyroid Activity	-88,49	-114,23	-82,93	-96,85
Magnesium Plasma	80,91	81,80	93,16	87,73
Macrophages Thymus	22,98	15,89	17,68	21,61
Creatinine Excretion	-16,21	-15,24	-14,60	-15,25
MxDMn as Vagal tone	-0,229	-0,015	-0,150	-0,261
0 Lymphocytes Blood	1,55	2,61	2,27	2,28
Phosphates Excretion	12,19	13,12	13,15	14,88
Macrophages Spleen	-9,45	-10,52	-10,60	-8,53
T helper Lymphocytes	3,39	1,85	3,48	4,06
Urea Plasma	-5,03	-8,21	-5,01	-4,78
Potassium Erythrocytes	7,07	7,06	6,73	7,33
Phosphates Plasma	-90,20	-96,01	-77,31	-89,24
Potassium Plasma	62,32	69,37	70,43	65,78
B Lymphocytes Blood	-2,62	-0,36	-1,30	-3,17
Entropy Splenocytogram	2911	2837	2755	2989
17-Ketosteroides Excretion	0,209	0,261	0,134	0,034
Corticosterone	-0,0231	0,0214	-0,0017	-0,0383
(Ca/K)^0,5 Plasma	329,6	340,8	300,6	317,1
Calcium Excretion	2,45	0,74	3,01	0,82
Leukocytes Blood	1,19	2,41	2,07	2,01
Plasmocytes Thymus	-6,59	-7,38	-7,98	-12,50
Microphages Spleen	5,91	8,49	7,26	4,39
Spleen Mass Index	0,05	0,12	0,09	0,31
Mineralocorticoid Activity	45,31	61,53	48,14	38,07
Sodium Excretion	0,076	0,140	0,085	0,042
Lymphoblastes Thymus	5,25	1,73	5,75	6,67
Uricosuria	-6,30	-8,80	-7,61	-6,41
Chloride Plasma	-30,20	-28,78	-31,51	-30,78
Sodium Plasma	40,36	39,21	41,74	40,75
Constant	-2895	-2954	-2944	-2981

Table 7. Classification matrix for uric acid metabolism clusters

Rows: observed classifications; columns: predicted classifications

Clusters	Percent Correct	S+Un+ p=,150	Sn+U-+ p=,317	SnUn+ p=,283	S-Un- p=,250
S+Un+	100	**9**	0	0	0
Sn+U-+	100	0	**19**	0	0
SnUn+	100	0	0	**17**	0
S-Un-	100	0	0	0	**15**
Total	100	9	19	17	15

CONCLUSION

So, the state of uric acid metabolism is closely related to the state of the neuro-endocrine-immune complex. This confirms both old [6,17,30] and modern [8,21] hypotheses about the physiological activity of uric acid.

According to the concept of our laboratory, this is due to the similarity molecule of the uric acid (**2,6,8**-tri**oxipurine**) with the molecules of theophylline (**2,6**-di**oxi**-1,3-dimethyl**purine** or 1,3-dimethyl**xantine**), caffeine (**2,6**-di**oxi**-1,3,7-trimethyl**purine** or 1,3,7-trimethyl**xantine**) and other methylxanthines, which, in turn, are structural homologues of adenosine [(2R,3R,4R,5R)-2-(6-amino**purine**-il)-5-(hydroximethyl) oxolan-3,4-diol)].

It is known that the effects of adenosine are realized through its receptors (A_1, A_{2A}, A_{2B}, A_3), which express virtually all populations of immunocytes such as T, NK, B lymphocytes, macrophages, neutrophils, dendritic and endothelial cells [1,15,16,32] as well as neurons of central and autonomous neural systems [7,8,18,21,22,29].

A detailed discussion will be conducted after the publication of the results of a similar study in humans.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the SR Institute of Medicine of Transport. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

REFERENCES

1. Apasov S, Chen JF, Smith P, Sitkovsky M. A_{2A} receptor dependent and A_{2A} receptor independent effects of extracellular adenosine on murine thymocytes in condicion of adenosine deaminase deficiency. Blood. 2000; 95(12): 3859-3867.
2. Baevsky RM, Berseneva AP. Use KARDIVAR system for determination of the stress level and estimation of the body adaptability. Standards of measurements and physiological interpretation. Moscow-Prague; 2008: 41.
3. Belousova OI, Fedotova MI. Comparative data on changes in spleen, thymus and bone marrow lymphocyte counts in the early post-irradiation period over a wide range of doses [in Russian]. Radiobiology-Radiotherapy. 1968; 9(3): 309-313.
4. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(1): 68-102.
5. Bombushkar IS, Gozhenko AI, Korda IV, Badiuk NS, Zukow W, Popovych IL. Features of the exchange of electrolytes and nitrogenous metabolites under different options of uric acid exchange in healthy female rats. Journal of Education, Health and Sport. 2020; 10(4): 405-415.
6. Chebanenko OI, Flyunt IS, Popovych IL, Balanovs'kyi VP, Lakhin PV. Water Naftussya and Water-salt Exchange [in Ukrainian]. Kyïv. Naukova dumka; 1997: 141.
7. Choudhury H, Chellappan DK, Sengupta P, Pandey M, Gorain B. Adenosine Receptors in Modulation of Central Nervous System Disorders. Curr Pharm Des. 2019;25(26):2808-2827.
8. El Ridi R, Tallima H. Physiological functions and pathogenic potential of uric acid: A review. J Adv Res. 2017; 8(5): 487-493.
9. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608.
10. Gozhenko AI, Smagliy VS, Korda IV, Zukow W, Popovych IL. Cluster analysis of uric acid exchange parameters in female rats. Journal of Education, Health and Sport. 2019; 9(11): 277-286.

11. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Popovych IL. Features of immune status in different states of uric acid metabolism in female rats. Journal of Education, Health and Sport. 2019; 9(12): 167-180.

12. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Popovych IL. Functional relationships between parameters of uric acid exchange and immunity in female rats. Actual problems of transport medicine. 2019; 4(58): 123–131.

13. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Relationships between parameters of uric acid exchange and immunity as well as microbiota in patients with neuroendocrine-immune complex dysfunction. Journal of Education, Health and Sport. 2020; 10(1): 165-175.

14. Gozhenko AI, Smagliy VS, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Relationships between changes in uric acid parameters metabolism and parameters of immunity and microbiota in patients with neuroendocrine-immune complex dysfunction. Journal of Education, Health and Sport. 2020; 10(2): 212-222.

15. Hoskin DW, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and NK cell function by adenosine and its contribution to immune evasion by tumor cells (Review). Int J Oncol. 2008; 32(3): 527-535.

16. Huang S, Apasov S, Koshiba M, SitkovskiM. Role of A_{2A} extracellular adenosine receptor mediated signaling in adenosine mediated inhibition of of T-cell activation and expansion. Blood. 1997; 90(4): 1600-1610.

17. Ivassivka SV, Popovych IL, Aksentiychuk BI, Flyunt IS. Physiological Activity of Uric Acid and its Role in the Mechanism of Action of Naftussya Water [in Ukrainian]. Kyïv: Computerpress; 2004: 163.

18. Jamwal S, Mittal A, Kumar P, Alhayani DM, Al-Aboudi A. Therapeutic Potential of Agonists and Antagonists of A1, A2a, A2b and A3 Adenosine Receptors. Curr Pharm Des. 2019; 25(26) :2892-2905.

19. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika; 1989: 78-138.

20. Kul'chyns'kyi AB, Kovbasnyuk MM, Kyjenko VM., Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 2. Correlations between parameters EEG, HRV and Phagocytosis. Journal of Education, Health and Sport. 2016; 6(10): 377-401.

21. Morelli M, Carta AR, Kachroo A, Schwarzschild A. Pathophysiological roles for purines: adenosine, caffeine and urate. Prog Brain Res. 2010; 183: 183-208.

22. Navalta JW, Fedor EA, Schafer MA, Lyons TS, Tibana RA, Pereira GB, Prestes J. Caffeine affects $CD8^+$ lymphocyte differently in naïve and familiar individuals following moderate intensity exercise. Int J Immunopathol Pharmacol. 2016; 29(2): 288-294.

23. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. Immune status, principles of its evaluation and correction of immune disorders [in Russian]. Kyiv: Zdorovya; 1995: 211.

24. Popadynets' OO, Gozhenko AI, Zukow W, Popovych IL. Relationships between the entropies of EEG, HRV, immunocytogram and leukocytogram. Journal of Education, Health and Sport. 2019; 9(5): 651-666.

25. Popovych IL, Bombushkar IS, Badiuk NS, Korda IV, Zukow W, Gozhenko AI. Features of the state of neuro-endocrine factors of adaptation under different options of uric acid metabolism in healthy female rats. Journal of Education, Health and Sport. 2020; 10(3): 352-362.

26. Popovych IL, Gozhenko AI, Bombushkar IS, Korda MM, Zukow W. Sexual dimorphism in relationships between of uricemia and some psycho-neuro-endocrine parameters. Journal of Education, Health and Sport. 2015; 5(5): 556-581.

27. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. Riga: Scholars' Press; 2020: 172.

28. Popovych IL, Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018; 8(2): 135-156.

29. Pousti A, Deemyad T, Malihi G. Mechanism of inhibitory effect of citalopram on isolated guinea-pig atria in relation to adenosine receptor. Hum Psychopharmacol. 2004; 19(5): 347-350.

30. Sofaer JA, Emery AF. Genes for super-intelligense? J Med Genet. 1981; 18: 410-413.
31. Smagliy VS, Gozhenko AI, Korda IV, Badiuk NS, Zukow W, Kovbasnyuk MM, Popovych IL. Variants of uric acid metabolism and their immune and microbiota accompaniments in patients with neuroendocrine-immune complex dysfunction. Actual problems of transport medicine. 2020; 1(59): 114–125.
32. Vigano S, Alatzoglou D, Irving M, Menetrier-Caux Ch, Caux Ch, Romero P, Coukos G. Targeting adenosine in cancer immunotherapy to enhance T-cell function. Front Immunol. 2019; 10: 925.