Kuchma Igor L., Gozhenko Anatoliy I., Flyunt Igor-Severyn S., Ruzhylo Sofiya V., Kovalchuk Galyna Y., Zukow Walery, Popovych Igor L. Role of the neuroendocrine complex in immunotropic effects of nitrogenous metabolites in rats. Journal of Education, Health and Sport. 2021;11(3):212-230. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2021.11.03.021
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2021.11.03.021
https://zenodo.org/record/4739899

Received: 05.03.2021. Revised: 14.03.2021. Accepted: 28.03.2021.

ROLE OF THE NEUROENDOCRINE COMPLEX IN IMMUNOTROPIC EFFECTS OF NITROGENOUS METABOLITES IN RATS

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Abstract

Background. We have previously shown that nitrogenous metabolites have immunomodulatory effects, both suppressor and enhancing, both in healthy rats and in humans exposed to pathogenic influences. The immunomodulatory effect of bilirubin is probably mediated through aryl hydrocarbon receptors, and uric acid through TL- and adenosine receptors of immune cells. The question of mediators of the immunomodulatory action of urea and creatinine remains open. We hypothesized the mediating role of mediators of the autonomic nervous system and adaptive hormones. The **aim** of this study is to analyze the relationships between the parameters of nitrogenous metabolites and the parameters of the autonomic nervous and endocrine systems, on the one hand, and between neuroendocrine and immune parameters - on the other hand. **Material and methods**. Experiment was performed on 60 healthy female Wistar rats. The plasma levels and urinary excretion of the nitrogenous metabolites, HRV and endocrine (corticosterone, triiodothyronine and testosterone plasma levels, calcitonin, parathyroid and mineralocorticoid activities, the thickness of glomerular, fascicular, reticular and medullar zones of adrenals) parameters as well as parameters of immunity were determined. **Results**. According to the results of canonical correlation analysis, the modulating effects of nitrogenous metabolites on neuroendocrine parameters are quite pronounced and almost identical in terms of bilirubin (R=0,603), creatinine (R=0,602), uric acid (R=0,599) and urea (R=0,586). Taken together, nitrogenous metabolites determine neuroendocrine parameters by 71,5% (R=0,845; $\chi^2_{(84)}$=179; p<10^{-6}). Triiodothyronine, fascicular and medullar areas of the adrenal glands, vagal tone and calcitonin activity were the

most susceptible to nitrogenous metabolites. In turn, neuroendocrine parameters determine the parameters of immunity, subject to exposure to nitrogenous metabolites, by 95,8% (R=0,979; $\chi^2_{(264)}$=405; p<10^{-6}). **Conclusion**. Previously identified immunomodulatory effects of nitrogenous metabolites are realized, perhaps, through the factors of the autonomic nervous and endocrine systems.
Key words: uric acid, creatinine, urea, bilirubin, neuro-endocrine parameters, relationships, rats.

INTRODUCTION

We have previously shown that the nitrogenous metabolites uric acid, bilirubin, creatinine, and urea exhibit immunotropic activity in both healthy rats [5,6,17] and humans exposed to pathogens [7,8,11,12,21]. The immunomodulatory effect of bilirubin is probably mediated through aryl hydrocarbon receptors, and uric acid through TL- and adenosine receptors of immune cells. The question of mediators of the immunomodulatory action of urea and creatinine remains open. We hypothesized the mediating role of mediators of the autonomic nervous system and adaptive hormones [17]. Our hypothesis is based on the concepts of functional-metabolic continuum [4] and neuroendocrine immunomodulation [9,16,18,22,23]. In testing the hypothesis in observations of people with post-radiation encephalopathy, we found links between nitrogenous metabolites and HRV markers of the autonomic nervous system - on the one hand, and between the latter and exactly the same immune parameters that are associated with nitrogenous metabolites - on the other hand [13].

The **aim** of this study is to analyze the relationships between the parameters of nitrogenous metabolites and the parameters of the autonomic nervous and endocrine systems, on the one hand, and between neuroendocrine and immune parameters - on the other hand.

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 variational swing (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [1].

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.

The plasma levels of the hormones of adaptation: corticosterone, triiodothyronine and testosterone (by the ELISA [10]) were determined.

Electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (flamming photometry) were determined in plasma and daily urine. The analyzes were carried out according to the instructions described in the manual [3].

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

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficient $(Cap \cdot Pu/Pp \cdot Cau)^{0,25}$, calcitonin by coefficient $(Cau \cdot Pu/Cap \cdot Pp)^{0,25}$ and mineralocorticoid by coefficient $(Nap \cdot Ku/Kp \cdot Nau)^{0,25}$, based on their classical effects and recommendations by IL Popovych [18].

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [2].

Methods for the determination of nitrogenous metabolites and immune parameters are given in the previous article [17].

Digital material is statistically processed on a computer using the software package "Statistica 20".

RESULTS AND DISCUSION

Screening of linear correlation coefficients between parameters of nitrogenous metabolites, on the one hand, and the recorded neuroendocrine parameters, on the other hand, revealed the following (Table 1).

In the next step of the analysis, a regression model was constructed for each plasma and urine nitrogenous metabolite by stepwise exclusion until the maximum level of adjusted R^2 was reached. As a result, it turned out that some regression models included parameters with an insignificant correlation coefficient, while some parameters with a significant correlation were outside the model.

Table 1. Correlation matrix for nitrogenous metabolites and neuroendocrine parameters

Variable	Cr Ex	Cr P	Urea Ex	UA Ex	Bilir	Urea P	UA P
MCA	0,19	0,07	-0,13	0,07	-0,19	0,28	-0,10
CTA	-0,24	0,19	0,21	-0,09	0,32	0,24	-0,30
PTA	-0,08	-0,33	-0,05	-0,01	-0,13	-0,13	-0,03
MxDMn	-0,11	-0,17	0,14	0,21	-0,06	-0,31	0,42
AMo	0,10	-0,01	-0,14	-0,10	-0,05	0,21	-0,29
Mode	-0,09	-0,12	0,14	0,19	0,08	-0,25	0,28
Corticosterone	0,02	0,51	0,02	-0,02	0,18	0,44	-0,21
Testosterone	-0,00	0,01	-0,06	-0,20	-0,20	0,11	-0,05
GlomerularZAC	-0,05	-0,12	-0,12	-0,19	-0,04	-0,10	-0,02
Fascicular ZAC	0,16	-0,16	-0,16	-0,45	-0,31	-0,10	-0,23
Reticular ZAC	0,08	0,13	-0,14	0,07	-0,29	0,12	-0,07
Medullar ZA	-0,26	-0,04	0,25	0,07	0,16	-0,23	0,14
Adrenals mass	0,03	0,04	0,14	-0,09	0,31	0,09	-0,10
T3	0,19	-0,10	-0,16	-0,47	-0,34	-0,00	-0,23

It is appropriate to start the analysis with those nitrogenous metabolites for which (at least to us) receptors on immunocytes are unknown. A stronger relationship was found between plasma creatinine and corticosterone levels (Fig. 1).

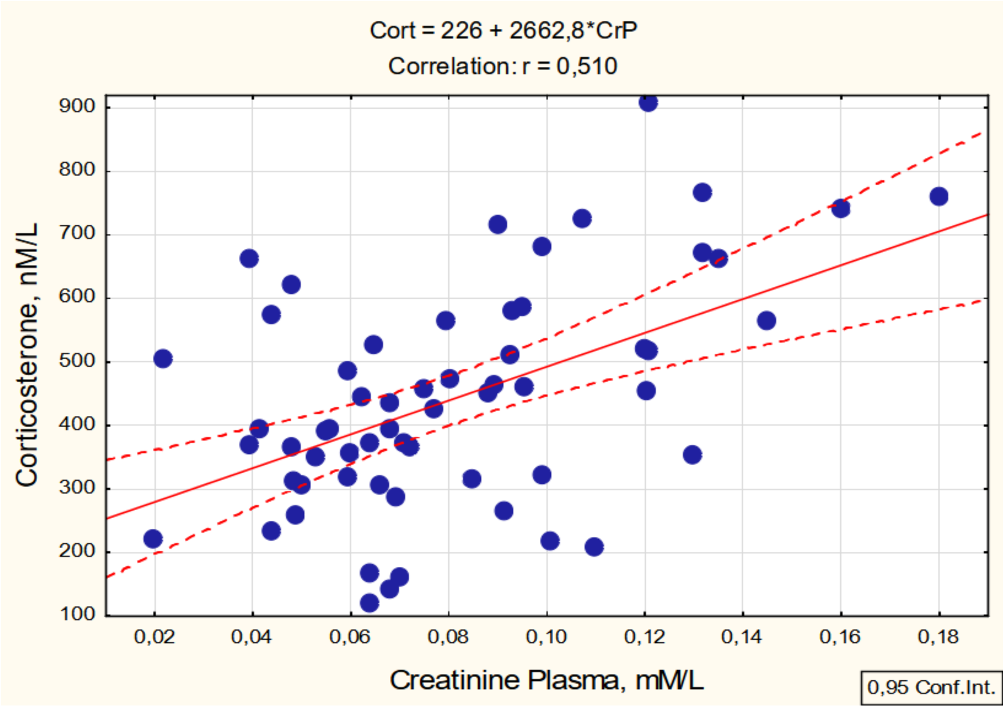


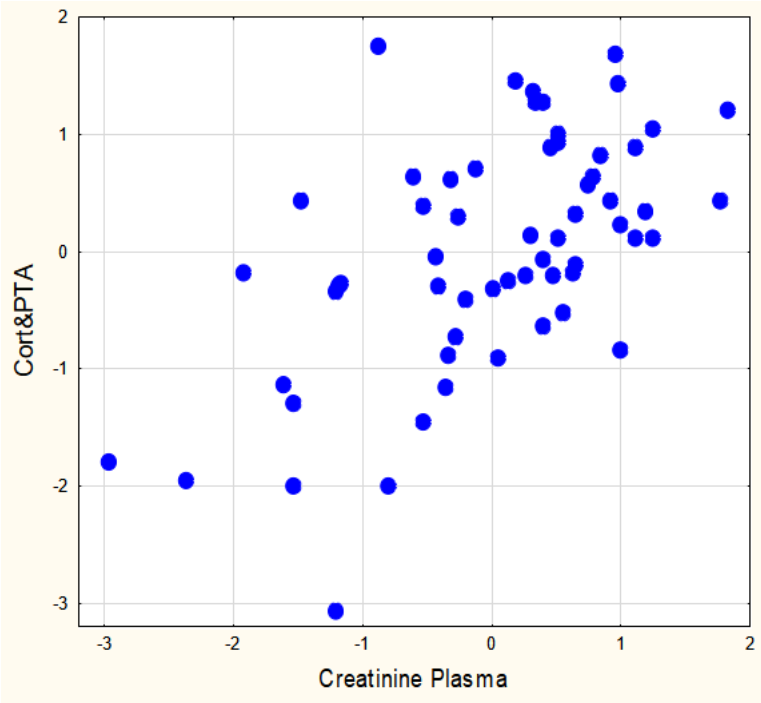
Fig. 1. Scatterplot of correlation between Creatinine (X-line) and Corticosterone (Y-line) Plasma in female rats

Creatinineemia is less associated with parathyroid activity. Both endocrine factors are determined by plasma creatinine by 32% (Table 2 and Fig. 2).

Table 2. Regression Summary for Creatinineemia

R=0,582; R^2=0,338; Adjusted R^2=0,318; $F_{(2,6)}$=14,6; p<10^{-5}

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(57)}$	p-level
Variables	r		Intercpt	0,083	0,020	4,15	10^{-3}
Corticosterone, nM/L	**0,51**	0,481	0,108	0,00009	0,00002	4,44	10^{-4}
Parathyroid Activity	**-0,33**	-0,281	0,108	-0,02359	0,00909	-2,59	0,012



R=0,582; R^2=0,338; $\chi^2_{(2)}$=23,5; p,10^{-5}; Λ Prime=0,662

Fig. 2. Scatterplot of canonical correlation between Creatininemia (X-line) and the Endocrine parameters (Y-line) in female rats

In contrast, creatinineuria is associated with endocrine factors weakly inverse, albeit statistically significantly (Table 3).

Table 3. Regression Summary for Creatinineuria

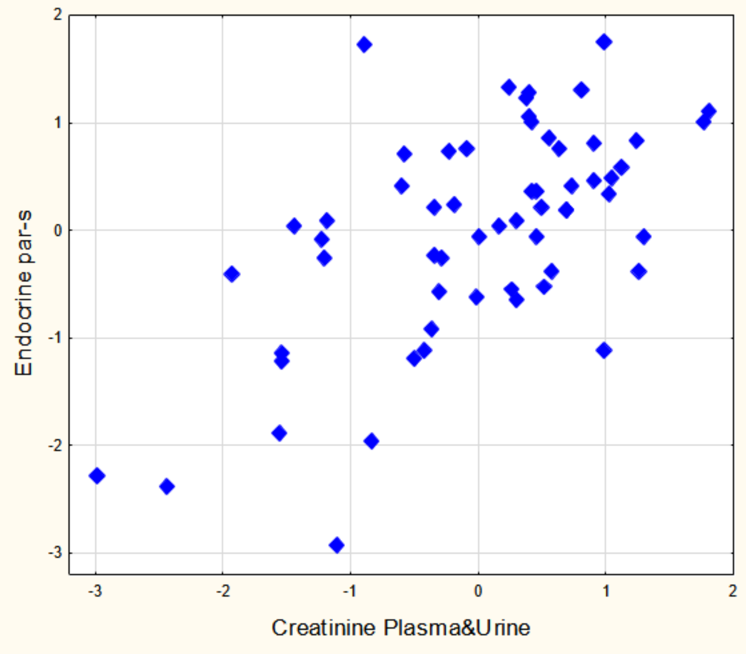
R=0,324; R^2=0,105; Adjusted R^2=0,073; $F_{(2,6)}$=3,3; p=0,043

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(57)}$	p-level
Variables	r		Intercpt	18,20	2,81	6,49	10^{-6}
Medullar ZA, µM	**-0,26**	-0,218	0,128	-0,0310	0,0182	-1,70	0,094
Calcitonin Activity	**-0,24**	-0,199	0,128	-2,4675	1,5909	-1,55	0,126

Canonical analysis shows that both creatinine exchange parameters determine the constellation of four endocrine parameters by 36% (Table 4 and Fig. 3).

Table 4. Factor load on canonical roots of Creatinine (left set) and Endocrine parameters (right set)

Left set	**Root 1**
Creatinineemia, mM/L	**-0,998**
Creatinineuria, µM/24h•100 g	-0,093
Right set	**Root 1**
Corticosterone, nM/L	**-0,845**
Calcitonin Activity	**-0,291**
Parathyroid Activity	**0,555**
Medullar Zone Adrenals, µM	**0,087**



R=0,602; R^2=0,363; $\chi^2_{(8)}$=31,6; p<10^{-4}; Λ Prime=0,565
Fig. 3. Scatterplot of canonical correlation between Creatinine (X-line) and the Endocrine parameters (Y-line) in female rats

Plasma urea levels are also most closely related to corticosterone (Fig. 4).

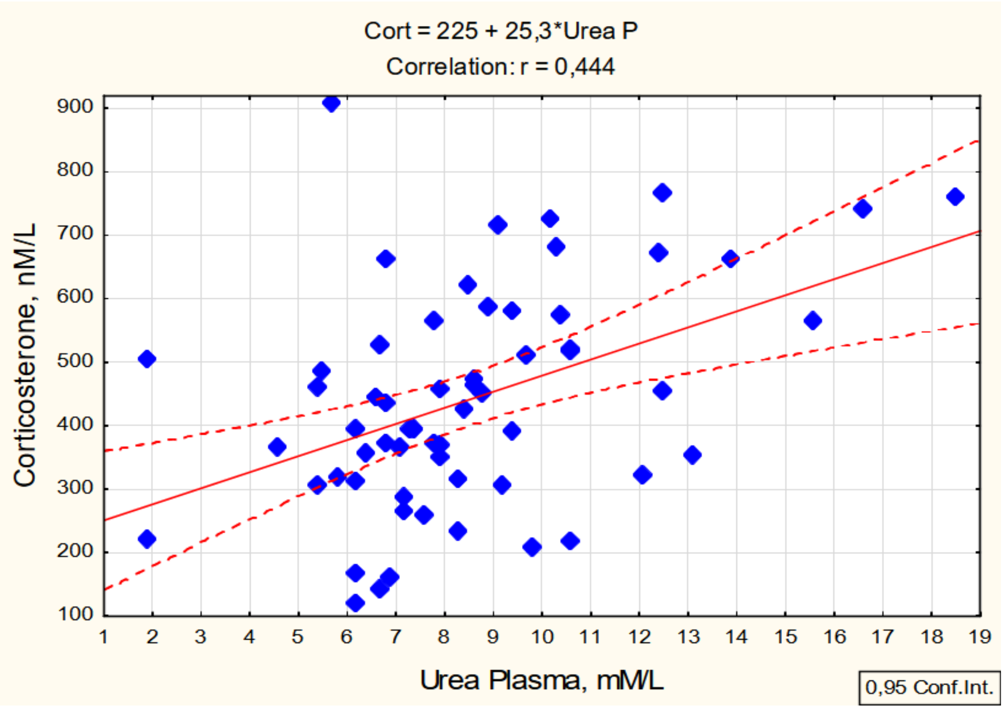


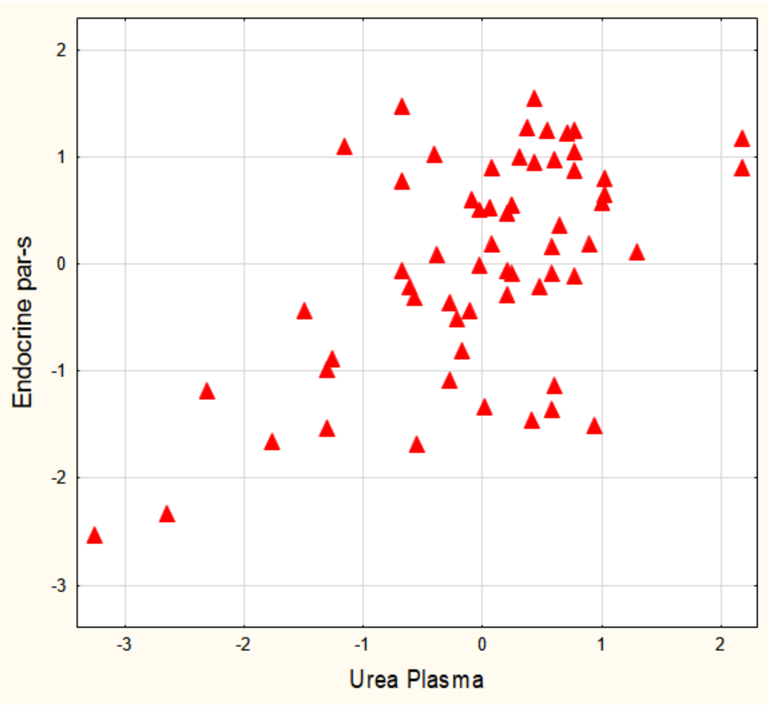
Fig. 4. Scatterplot of correlation between Urea (X-line) and Corticosterone (Y-line) Plasma in female rats

Weaker positive correlation was found for mineralocorticoid and calcitonin activities, while negative - with the thickness of the adrenal medulla (source of circulating catecholamines), as well as with with the Mode HRV (r = -0,25), which is their inverse reflection, and the vagal tone (r = -0,31). However, the last two parameters after the step-by-step exclusion turned out to be outside the regression model for some reason (Table 5 and Fig. 5).

Table 5. Regression Summary for Urea Plasma

R=0,568; R^2=0,323; Adjusted R^2=0,273; $F_{(4,6)}$=6,6; p=0,0002

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	2,22	2,89	0,77	0,446
Corticosterone, nM/L	**0,44**	0,407	0,116	0,007	0,002	3,52	0,001
Mineralocorticoid Activity	**0,28**	0,195	0,155	0,635	0,506	1,25	0,215
Calcitonin Activity	**0,24**	0,204	0,117	1,611	0,927	1,74	0,088
Medullar ZA, μM	**-0,23**	-0,172	0,156	-0,016	0,014	-1,10	0,277



R=0,568; R^2=0,323; $\chi^2_{(4)}$=21,8; p=0,0002; Λ Prime=0,677

Fig. 5. Scatterplot of canonical correlation between Urea Plasma (X-line) and the Endocrine parameters (Y-line) in female rats

Urea excretion, like creatinine, is also weakly associated with endocrine factors, and statistically insignificant (Table 6).

Table 6. Regression Summary for Urea Excretion

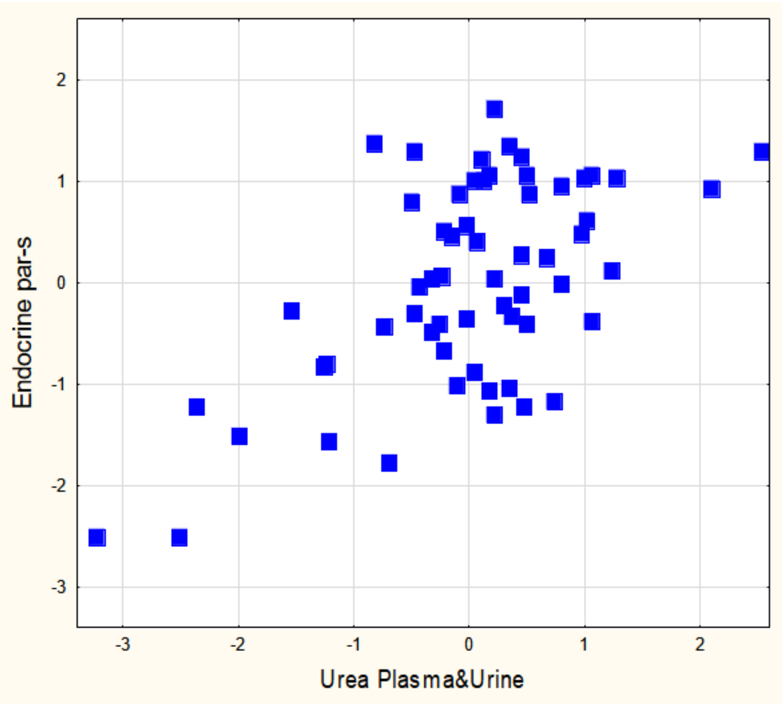
R=0,299; R^2=0,089; Adjusted R^2=0,057; $F_{(2,6)}$=2,8; p=0,069

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	-1,45	102	-0,01	0,989
Medullar ZA, μM	**0,25**	0,214	0,129	1,102	0,665	1,66	0,103
Calcitonin Activity	**0,21**	0,169	0,129	75,96	57,98	1,31	0,195

As a result, the determining effect of urea on this endocrine constellation was almost similar to that of creatinine: 34,3% vs 36,3% (Table 7 and Fig. 6).

Table 7. Factor load on canonical roots of Urea (left set) and Endocrine parameters (right set)

Left set	Root 1
Urea Plasma, mM/L	**-0,958**
Urea Excretion, µM/24h•100 g	**0,127**
Right set	**Root 1**
Corticosterone, nM/L	**-0,751**
Mineralocorticoid Activity	**-0,544**
Calcitonin Activity	**-0,304**
Medullar Zone Adrenals, µM	**0,513**



R=0,586; R^2=0,343; $\chi^2_{(8)}$=28,7; p=0,0004; Λ Prime=0,596

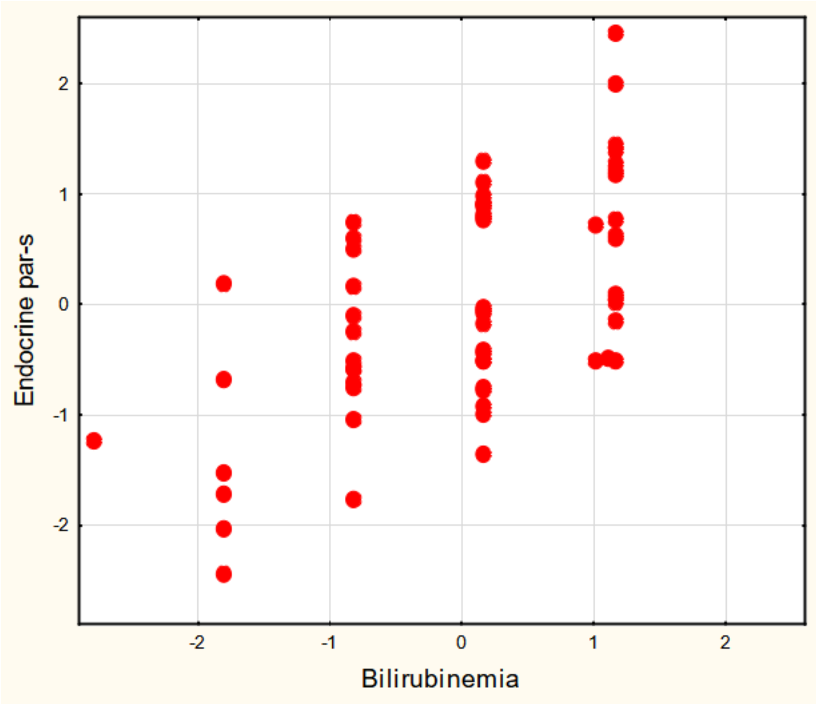
Fig. 6. Scatterplot of canonical correlation between Urea (X-line) and the Endocrine parameters (Y-line) in female rats

Interestingly, a similar measure of determination (36,3%) of endocrine parameters is also demonstrated by plasma bilirubin (Table 8 and Fig. 7).

Table 8. Regression Summary for Bilirubinemia

R=0,603; R^2=0,363; Adjusted R^2=0,291; $F_{(6,5)}$=5,0; p=0,0004

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(53)}$	p-level
Variables	r		Intercpt	3,48	2,36	1,47	0,147
Triiodothyronine, nM/L	**-0,34**	-0,214	0,120	-1,137	0,637	-1,78	0,080
Reticular ZAC, µM	**-0,29**	-0,211	0,119	-0,044	0,025	-1,77	0,082
Testosterone, nM/L	**-0,20**	-0,242	0,117	-0,256	0,124	-2,07	0,043
Calcitonin Activity	**0,32**	0,126	0,120	0,695	0,660	1,05	0,297
Adrenals Mass, mg/100 g	**0,31**	0,342	0,115	16,97	5,69	2,98	0,004
Corticosterone, nM/L	**0,18**	0,192	0,116	0,0023	0,0014	1,66	0,103



R=0,603; R^2=0,363; $\chi^2_{(6)}$=24,8; p=0,0004; Λ Prime=0,637

Fig. 7. Scatterplot of canonical correlation between Bilirubin Plasma (X-line) and the Endocrine parameters (Y-line) in female rats

In this case, bilirubin upregulates calcitonin activity, adrenal mass and plasma corticosterone levels, while downregulates the secretion of testosterone (in females!) by the adrenal reticular zone, as well as plasma levels of triiodothyronine.

Plasma uric acid levels are positively correlated with vagal tone (Fig. 8), while inversely with calcitonin activity and plasma triiodothyronine levels (Table 9), as well as sympathetic tone not included in the model (r = -0,29). The degree of determination is 25% (Fig. 9).

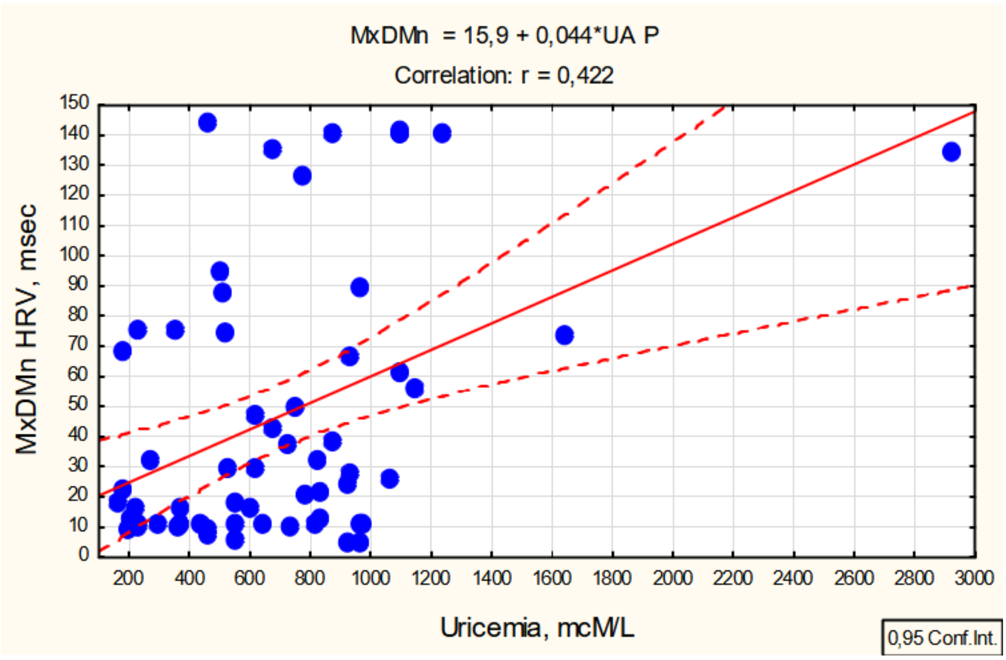
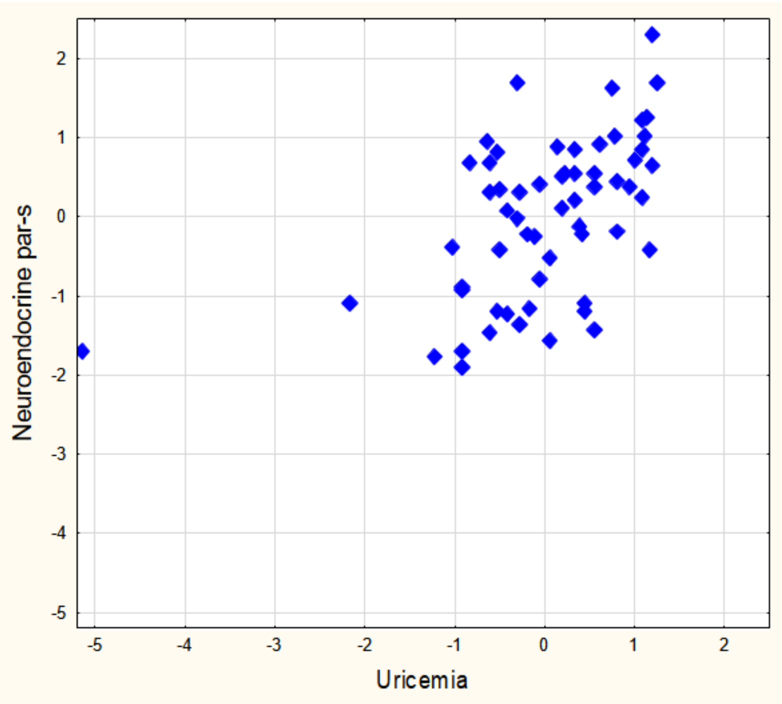


Fig. 8. Scatterplot of correlation between Uricemia (X-line) and MxDMn HRV (Y-line) in female rats

Table 9. Regression Summary for Uricemia

$R=0,535$; $R^2=0,287$; Adjusted $R^2=0,248$; $F_{(3,6)}=7,5$; $p=0,0003$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(56)}$	p-level
Variables	r		Intercpt	1585	408	3,89	0,0003
MxDMn HRV, msec	**0,42**	0,356	0,117	3,415	1,122	3,04	0,0035
Calcitonin Activity	**-0,30**	-0,310	0,115	-346,7	129,2	-2,68	0,0096
Triiodothyronine, nM/L	**-0,23**	-0,197	0,119	-212,5	127,8	-1,66	0,1019



$R=0,535$; $R^2=0,287$; $\chi^2_{(3)}=19,1$; $p=0,0003$; Λ Prime=0,713

Fig. 9. Scatterplot of canonical correlation between Uricemia (X-line) and the Neuroendocrine parameters (Y-line) in female rats

Uricosuria is also associated with the level of triiodothyronine inversely, but much more closely (Fig. 10), as well as with the thickness of the fascicular zone of the adrenal cortex (Fig. 11). In the regression model, the program also included the thickness of the glomerular zone and testosteroneemia (Table 10). This endocrine constellation is determined by uricosuria by 24% (Fig. 12).

Both parameters of uric acid exchange, taken together, determine the constellation of six neuroendocrine parameters by 36% (Table 11 and Fig. 13).

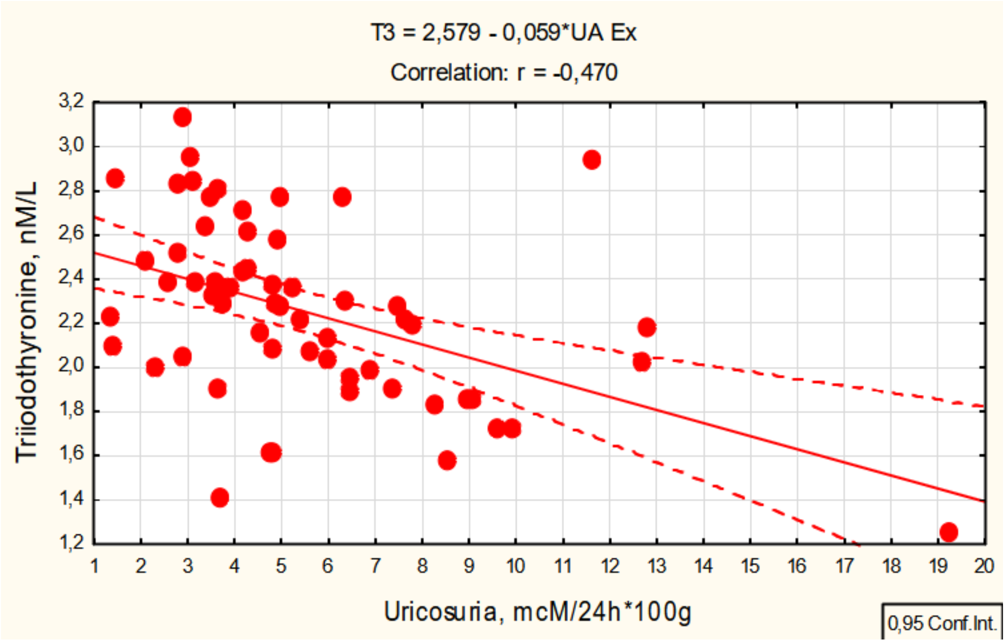


Fig. 10. Scatterplot of correlation between Uricosuria (X-line) and Triiodothyronine (Y-line) in female rats

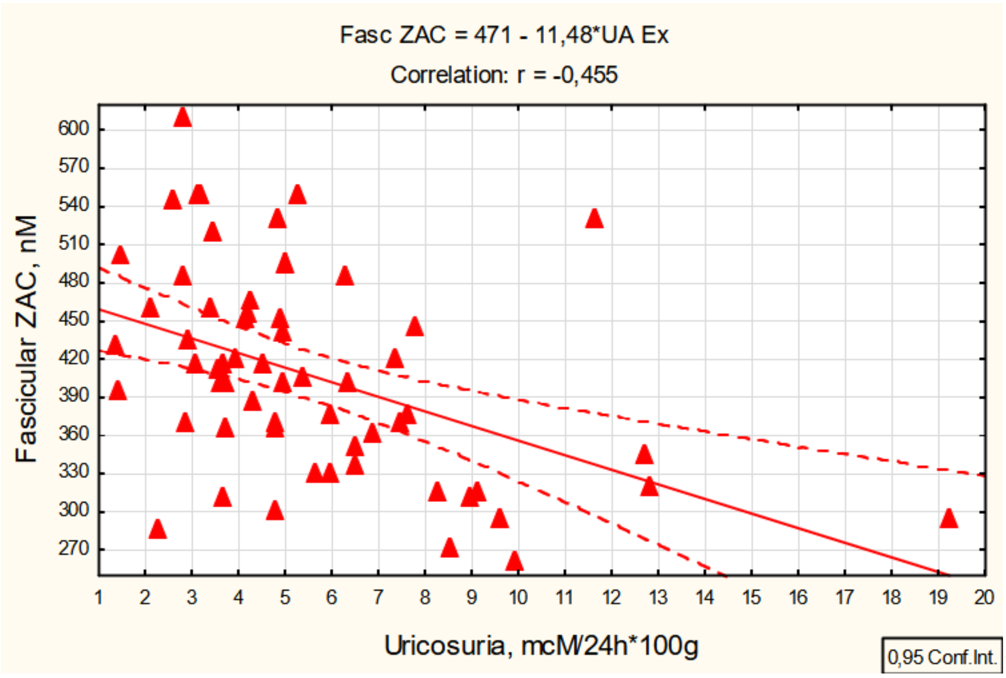
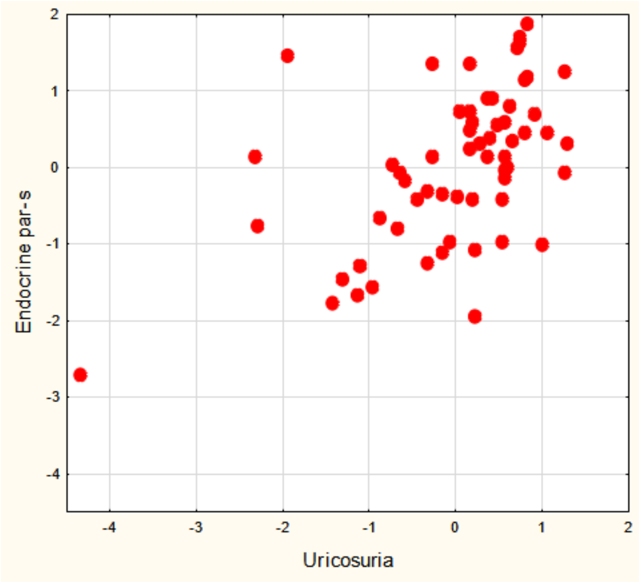


Fig. 11. Scatterplot of correlation between Uricosuria (X-line) and the thickness of Fascicular zone adrenal cortex (Y-line) in female rats

Table 10. Regression Summary for Uricosuria

R=0,540; R^2=0,291; Adjusted R^2=0,240; $F_{(4,6)}$=5,7; p=0,0007

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	17,51	2,75	6,36	10^{-6}
Triiodothyronine, nM/L	**-0,47**	-0,295	0,148	-2,334	1,175	-1,99	0,052
Fascicular ZAC, µM	**-0,45**	-0,220	0,151	-0,0087	0,0060	-1,46	0,150
Testosterone, nM/L	**-0,20**	-0,132	0,117	-0,208	0,184	-1,13	0,264
Glomerular ZAC, µM	**-0,19**	-0,140	0,117	-0,0118	0,0098	-1,20	0,236

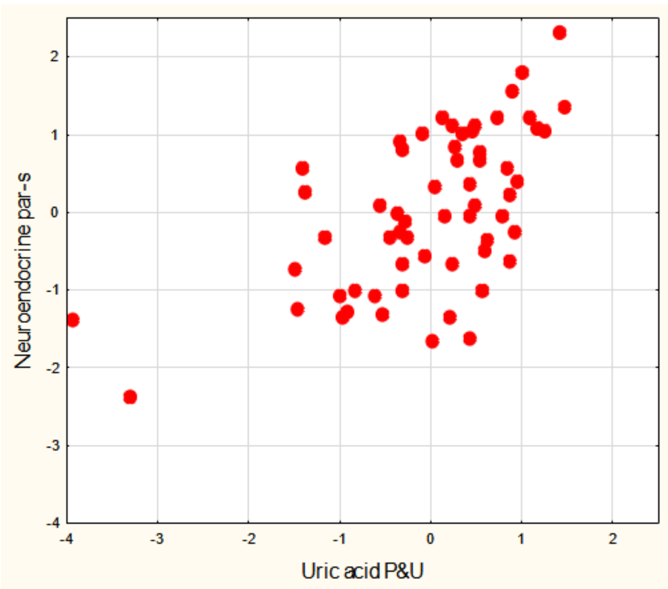


R=0,540; R^2=0,291; $\chi^2_{(4)}$=19,3; p=0,0007; Λ Prime=0,709

Fig. 12. Scatterplot of canonical correlation between Uricosuria (X-line) and the Endocrine parameters (Y-line) in female rats

Table 11. Factor load on canonical roots of Uric acid (left set) and Neuroendocrine parameters (right set)

Left set	**Root 1**
Uricosuria, μM/24h•100 g	**-0,918**
Uricemia, μM/L	**-0,786**
Right set	**Root 1**
Triiodothyronine, nM/L	**0,723**
Fascicular ZAC, μM	**0,704**
Calcitonin Activity	**0,330**
Testosterone, nM/L	**0,276**
Glomerular ZAC, μM	**0,231**
MxDMn HRV, msec	**-0,564**



R=0,599; R^2=0,359; $\chi^2_{(12)}$=35,7; p=0,0004; Λ Prime=0,519

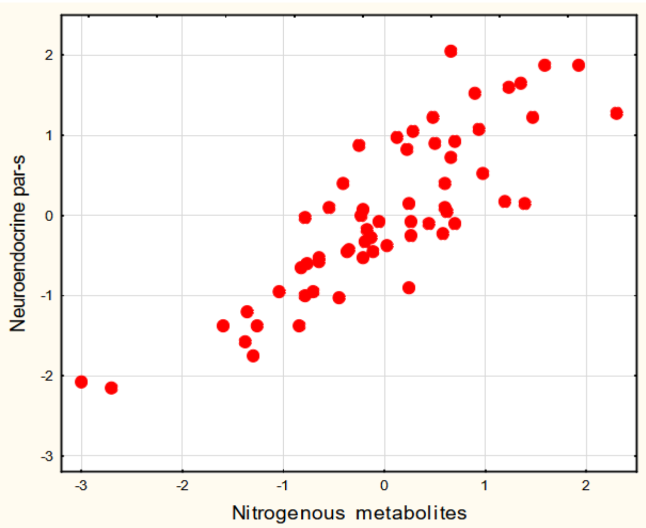
Fig. 13. Scatterplot of canonical correlation between Uric acid (X-line) and the Neuroendocrine parameters (Y-line) in female rats

As a result of canonical correlation analysis involving all registered nitrogenous metabolites, on the one hand, and neuroendocrine parameters, on the other hand, two pairs of canonical roots were formed.

The nitrogenous root of the first pair receives the maximum factor load from uricosuria and less load from bilirubinemia, uricemia and urea excretion, as well as inversely from creatinine excretion. The neuroendocrine root represents the parameters subject to **upregulation** by creatinineuria while **downregulation** by other nitrogenous metabolites. This neuroendocrine constellation is determined by the corresponding nitrogen constellation by 71,5% (Fig. 14).

Table 12. Factor load on first canonical roots of nitrogenous metabolites (left set) and neuroendocrine parameters (right set)

Left set	Root 1
Uricosuria, μM/24h•100 g	-0,521
Bilirubinemia, μM/L	-0,371
Urea Excretion, μM/24h•100 g	-0,332
Uricemia, μM/L	-0,281
Creatinineuria, μM/24h•100 g	0,360
Right set	**Root 1**
Medullar ZA, μM	-0,598
MxDMn HRV, msec	-0,430
Calcitonin Activity	-0,404
Triiodothyronine, nM/L	0,782
Fascicular ZAC, μM	0,706
Mineralocorticoid Activity	0,321
Testosterone, nM/L	0,254
Reticular ZAC, μM	0,243
Glomerular ZAC, μM	0,168



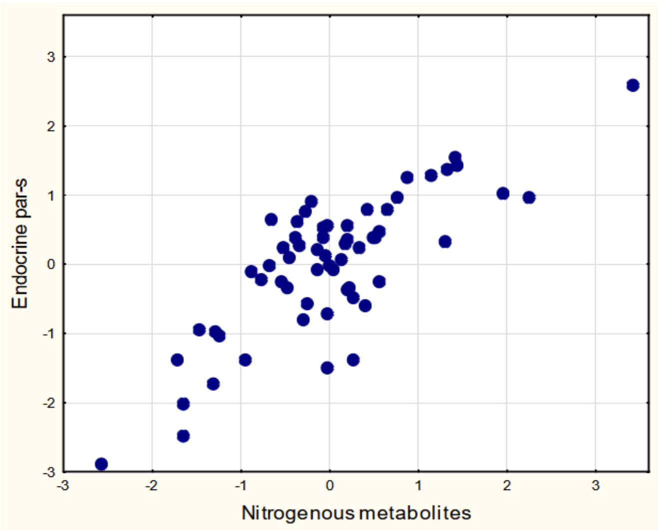
$R=0,845$; $R^2=0,715$; $\chi^2_{(84)}=179$; $p<10^{-6}$; Λ Prime=0,026

Fig. 14. Scatterplot of canonical correlation between the nitrogenous metabolites (X-line) and neuroendocrine parameters (Y-line) in female rats. First pair of Roots

The second pair of roots is poorly structured and illustrates the relationship between other nitrogen-endocrine constellations (Table 13 and Fig. 15).

Table 13. Factor load on second canonical roots of nitrogenous metabolites (left set) and endocrine parameters (right set)

Left set	Root 2
Bilirubinemia, μM/L	**0,359**
Creatininemia, mM/L	**0,189**
Urea Excretion, μM/24h•100 g	**0,187**
Uricosuria, μM/24h•100 g	**-0,326**
Urea Plasma, mM/L	**-0,184**
Right set	**Root 2**
Medullar ZA, μM	**0,294**
Fascicular ZAC, μM	**0,208**
Corticosterone, nM/L	**0,198**
Triiodothyronine, nM/L	**0,190**
Adrenals Mass, mg/100 g	**0,157**
Parathyroid Activity	**-0,497**
Mineralocorticoid Activity	**-0,482**
Reticular ZAC, μM	**-0,179**



R=0,796; R^2=0,634; $\chi^2_{(66)}$=117; p<10^{-4}; Λ Prime=0,092

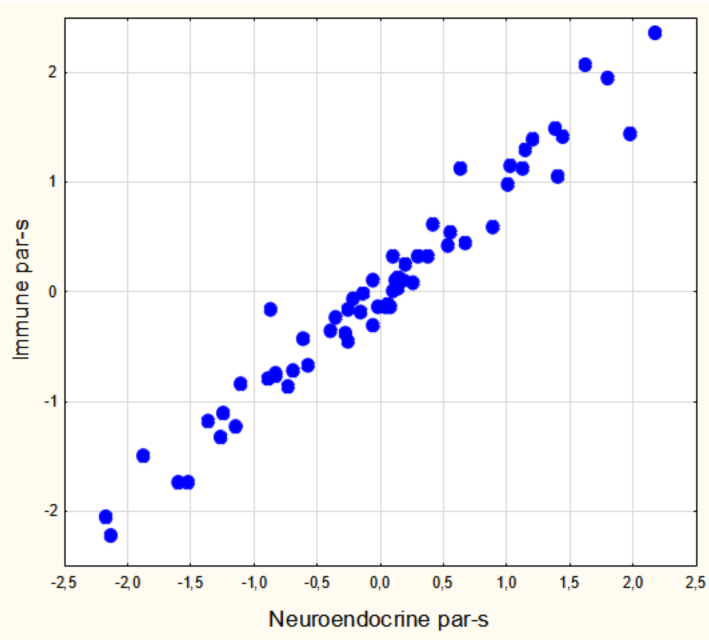
Fig. 15. Scatterplot of canonical correlation between the nitrogenous metabolites (X-line) and endocrine parameters (Y-line) in female rats. Second pair of Roots

At the final stage of the analysis the connections between neuroendocrine parameters and those parameters of immunity which in the previous research were revealed subject to modulating influence of nitrogenous metabolites are found out.

Two neuroendocrine-immune pairs of canonical roots are formed. The first pair of roots reflects the immunomodulatory effect, primarily of triiodothyronine and glucocorticoids, to a lesser extent - mineralocorticoids, androgens and parathyroid hormone, as well as, conversely, catecholamines, vagus and calcitonin (Table 14). The degree of determination is 96% (Fig. 16).

Table 14. Factor load on first canonical roots of neuroendocrine (left set) and immune parameters (right set)

Left set	Root 1
Triiodothyronine, nM/L	**0,967**
Fascicular ZAC, µM	**0,634**
Mineralocorticoid Activity	**0,329**
Reticular ZAC, µM	**0,304**
Parathyroid Activity	**0,229**
Testosterone, nM/L	**0,163**
Medullar Zone Adrenals, µM	**-0,403**
MxDMn HRV, msec	**-0,385**
Calcitonin Activity	**-0,318**
Right set	**Root 1**
Natural Killers Blood, %	**0,923**
Monocytes Blood, %	**0,906**
Phagocytic Index Monocytes, %	**0,248**
Reticulocytes Thymus, %	**0,232**
Hassal's corpuscles Thymus, %	**0,150**
Fibroblastes Spleen, %	**0,139**
Stub Neutrophils Blood, %	**0,104**
Eosinophiles Spleen, %	**0,093**
Spleen Mass Index, g/100g	**0,050**
Microbial Count Neutrophils	**-0,902**
Phagocytic Index Neutrophils, %	**-0,642**
Lymphoblastes Spleen, %	**-0,378**
Lymphocytes Thymus, %	**-0,260**
Lymphoblastes Thymus, %	**-0,232**
Th Lymphocytes Blood, %	**-0,171**
Entropy Splenocytogram	**-0,167**
Macrophages Thymus, %	**-0,098**



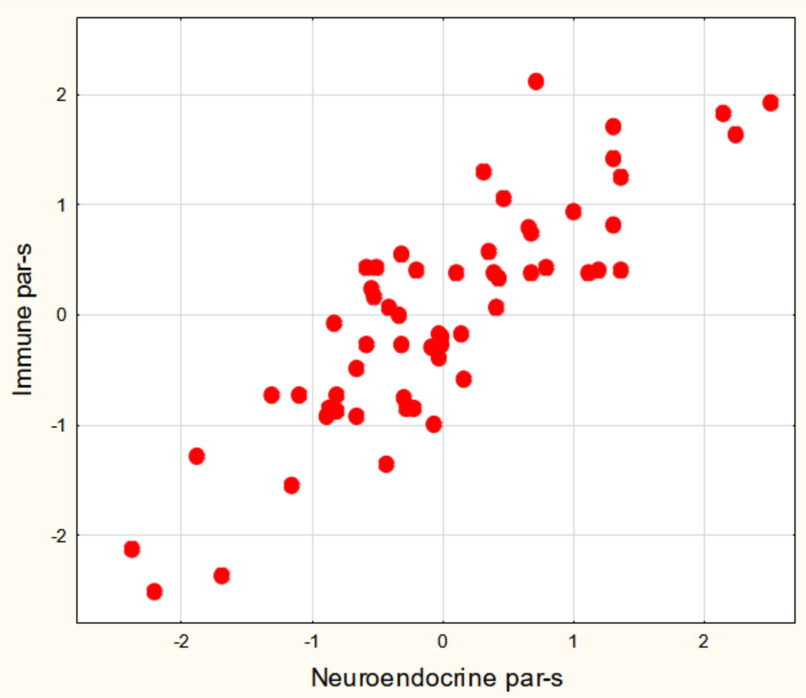
R=0,979; R^2=0,958; $\chi^2_{(264)}$=405; $p<10^{-6}$; Λ Prime<10^{-4}

Fig. 16. Scatterplot of canonical correlation between the neuroendocrine (X-line) and immune (Y-line) parameters in female rats. First pair of Roots

The second neuroendocrine root is poorly structured and reflects the modulating effect of hormones and vagus on another constellation of immune parameters (Table 15 and Fig. 17).

Table 15. Factor load on second canonical roots of neuroendocrine (left set) and immune parameters (right set)

Left set	Root 2
Medullar Zone Adrenals, µM	**0,424**
Fascicular ZAC, µM	**0,357**
Testosterone, nM/L	**0,334**
Corticosterone, nM/L	**0,138**
Glomerular ZAC, µM	**0,110**
MxDMn HRV, msec	**-0,373**
Parathyroid Activity	**-0,315**
Mineralocorticoid Activity	**-0,301**
Reticular ZAC, µM	**-0,238**
Right set	**Root 2**
Entropy Leukocytogram	**-0,557**
Endotheliocytes Thymus, %	**-0,419**
Microphages Spleen, %	**-0,381**
Fibroblastes Spleen, %	**-0,227**
Phagocytic Index Neutrophils, %	**-0,215**
Phagocytic Index Monocytes, %	**-0,143**
Leukocytes Blood, 10^9/L	**0,202**
Eosinophiles Spleen, %	**0,186**
Th Lymphocytes Blood, %	**0,164**
Eosinophiles Blood, %	**0,133**
Reticulocytes Thymus, %	**0,115**
Stub Neutrophils Blood, %	**0,111**
Macrophages Thymus, %	**0,103**



R=0,854; R^2=0,729; $\chi^2_{(231)}$=274; p=0,029; Λ Prime=0,0014

Fig. 17. Scatterplot of canonical correlation between the neuroendocrine (X-line) and immune (Y-line) parameters in female rats. Second pair of Roots

It seems that nitrogenous metabolites modulate the activity of the autonomic nervous system, as well as the adrenal, thyroid and parathyroid glands, mediators and hormones which, in turn, have an immunomodulatory effect. This assumption is consistent with the concepts of functional-metabolic continuum [4] and neuroendocrine-immune complex [16,18,22,23].

However, the question of the role of the central nervous system in the immunotropic effects of nitrogenous metabolites in line with the concept of the immune homunculus [14,15,19,20,24,25,26] remains open, which will be the subject of our next research.

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 Ukrainian Scientific Research Institute for 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).

Conflict of Interest. The authors declare that there is no conflict of interest that could be perceived as interfering with publication of the article.

Competing Interests. The authors declare that they have no competing interests.

Funding sources. This study has not received any financial support from any government, community or commercial organization.

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