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Structural Changes of Endocrine Juxtaglomerular Complex of the Kidney Caused by the Combined Effect of the Man–Made Environmental Factors.

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The analysis of morphometric indices and ultrastructural characteristics of the components of the renal endocrine juxtaglomerular complex (juxtaglomerular cells and macula densa cells) in 80 rats and mice kept in different parts of the Carpathians for two and four months has been done. The trend to increasing diameters and volumes of cells and cytoplasm while reducing nuclear-cytoplasmic index of juxtaglomerular cells of all types of granularity and macula densa cells of animals, kept in bad ecological regions, was mentioned: chemically polluted towns (Kalush and Burshtyn), increased radiological control zone – the village of Stetseva (Snyatyn region). The most significant changes in morphometric indices and activation of biosynthetic processes in cells (granularity of type II) were found. Cells (granularity of type III) containing a large number of secretory granules and macula densa cells were found to be the least sensitive sells to the anthropogenic environmental factors. The dependence of morphometric indices and ultrastructural characteristics of endocrine juxtaglomerular cells and macula densa sells of the animals' kidneys (control group) on the degree of pollution and the duration of the number of anthropogenic factors (different regions of Carpathians) was proved. The data may be considered as the result of adaptive changes of renal endocrine juxtaglomerular complex by man–made pollution effect.

Keyword: Renal Juxtaglomerular Complex, Morphometric Indices, Man–Made Environmental Factors.

1. Introduction

Taking into account the progressing technological environment denaturation the study of peculiarities of the human body's adaptation to the combined effect of low–intensity hazards of pollution became the priory modern trend of medicine [1]. Kidneys play an important role in supplying the compensatory-adaptive reactions to the effects of xenobiotics taken by the body, but not accumulated in certain organs and tissues. [2,3]. They maintain homeostasis of the body by means of neuro-humoral mechanisms of its regulation. Among the structural components implementing these mechanisms the juxtaglomerular complex (JGC) plays an important role. It is the morphological substrate of the renin–angiotensin system [4, 5, 6].

Our previous studies have defined the morphometric indices of endocrine juxtaglomerular (JG) cells, and peculiarity of their distribution in different renal vessels (interlobular arteries, glomerular arterioles) and macula densa cells in normal [7]. The taken results were basic ones for studying the influence of man–made pollution on the renal endocrine system, including the JGC.

2. The Aim of Study:

To study the dynamics of changes of morphometric indices of juxtaglomerular cells and macula densa cells of white rats and mice influenced by a combination of man–made environmental factors.

3. Materials and Methods:

The research was conducted on 40 white rats and 40 white mice kept for two or four months in different regions of the Carpathians: Kosiv – positive ecological region, chemically polluted towns – Kalush and Burshtyn, increased radiological control region – the village of Stetseva (Snyatyn region). The diet of animals consisted of local ordinary food and local natural water. Care of the rats and mice and all manipulations having been done were carried out strictly following the ethical and legal standards and requirements of the Law of Ukraine "Protection of animals" (from February, 21st, 2006). Euthanasia of animals was carried out using an overdose of ether as an anesthetic.

For light optical and electronomicroscopic study the drugs were prepared using conventional methods. 50 JG cells and 50 macula densa cells of each animal (control group) were analyzed. Large and small diameter of JG cells, macula densa sells and their nuclei were determined by means of the microscope Micros Austria (Camera DCM 900) having used the program Biovision 4.1, West Medica HandlesGmbH Company.

To determine the statistical significance (P), correlations between indices, computer program "Excel" was used (Microsoft Office).

4. Results and Discussion:

Taking into account our previous data connected with bio indication of pollution intensity [8, 9], morphometric indices of JG cells and macula densa cells of animals kept in Kosiv (the Carpathians) were taken as control data. Studying the changes of JG cells the parameters of every three types depending on the degree of granularity were calculated: I – there are few granules around the nucleus (very small amount), II – granules are diffusely located in the cytoplasm (small amount), III – cytoplasm is densely filled with granules (large amount) respectively. The data of large and small diameters of JG cells of white rats kept for two months in control region were: type I – (10,14 ± 9,31) and (7,33 ± 0,44), II – (10,74 ± 0,57) and (8,25 ± 0,63) and III – (11,62 ± 0,92) and (8,89 ± 0,76) mkm respectively. During the fourth month the experiment showed little changes in JG cells' diameters of all types. Nuclei's volumes were increased and cytoplasm's and cells' volumes were decreased. Nuclear-cytoplasmic index of JG cells was increased by 3.85% (Type I) and 4.35% (Type II) respectively, (Type III) – no changes (Table 1).

Table 1: Morphometric indices of juxtaglomerular cells of rats kept in different regions of the Carpathians for two (A) and for four (B) months, M ± m

Indices, term of experiment	Types of granularity of juxtaglomerulocytes		
	I	II	III
Kosiv			
Volume of a cell, mkm^3 A	402,85±41,06	467,92±38,64	678,67±50,07
B	398,67±53,20	453,48±29,42	659,42±34,79
Volume of a nucleus, mkm^3 A	83,54±9,46	86,9 5±14,91	88,64±17,05
B	85,72±12,50	88,03±16,73	89,37±14,86
Volume of cytoplasm, mkm^3 A	321,28±42,14	382,14±39,23	589,21±45,28
B	310,51±49,36	365,96±28,47	576,68±33,56
Nuclear-cytoplasmic index s.u. A	0,26±0,04	0,23±0,02	0,15±0,03
B	0,27±0,03	0,24±0,04	0,15±0,02
Kalush			
Volume of a cell, mkm^3 A	459,52±39,12*	536,22±42,36*	699,56±29,78
B	474,38±28,65*	581,96±50,9*	720,14±40,83
Volume of a nucleus, mkm^3 A	84,58±15,26	86,17±13,35	87,42±18,54
B	85,89±19,32	84,96±11,09	88,63±9,98

Volume of cytoplasm, mkm^3	A	373,46±34,97*	448,29±40,23*	611,97±32,59
	B	387,90±25,36*	495,64±47,69*	630,86±38,44
Nuclear-cytoplasmic index, s.u.	A	0,23±0,03*	0,19±0,02*	0,15±0,03
	B	0,22±0,04*	0,17±0,03*^{o}	0,14±0,02
Burshtyn				
Volume of a cell, mkm^3	A	482,76±41,06*	568,89±38,64*	706,94±50,07
	B	505,18±53,20*	610,32±29,42*	733,86±34,39
Volume of a nucleus, mkm^3	A	83,96±6,87	82,6 5±7,42	86,31±15,59
	B	84,78±10,51	85,44±13,06	85,77±11,43
Volume of cytoplasm, mkm^3	A	398,95±38,26*	481,26±35,90*	623,69±47,81
	B	419,32±46,91*	524,09±28,73*	647,25±33,50
Nuclear-cytoplasmic index, s.u.	A	0,21±0,04*	0,17±0,05*	0,14±0,04
	B	0,20±0,03*	0,16±0,04*	0,13±0,03*
Stetseva				
Volume of a cell mkm^3	A	448,15±41,06*	531,04±50,29*	702,60±35,07
	B	477,07±53,20*	579,21±28,43*	718,34±45,89
Volume of a nucleus, mkm^3	A	82,59±5,46	82,4 5±14,91	83,24±17,05
	B	84,12±7,30	83,90±16,73	84,87±14,86
Volume of cytoplasm mkm^3	A	364,92±36,58*	443,17±47,53*	617,81±31,26
	B	390,67±40,18*	499,74±19,40*	625,63±42,38
Nuclear-cytoplasmic index, s.u.	A	0,23±0,04*	0,19±0,02*	0,14±0,03
	B	0,22±0,03*	0,17±0,04*^{o}	0,14±0,02

Notes: 1. * – Probability of difference compared to control group ($p < 0,05$).
2. o – probability of difference between indices of two or four month periods ($p < 0,05$).

In the kidneys of rats, kept for two months in chemically polluted town (Kalush), large and small diameters of cells (all three types) were increased and were: I – (10,69 ± 0,72) and (7,61 ± 0,44), II – (11,03 ± 0,62) and (8,32 ± 0,70), III – (12,00 ± 0,84) and (9,15 ± 0,39) mm respectively. The volume of these cells was also increased respectively by 14.07, 14.60 and 3.08% compared to the control group. Changes of the volume of cytoplasm were almost the same. Nuclear-cytoplasmic index of JG cells (Types I, II) was decreased by 13.04 and 21.05% compared to the control group, and cells (type III) were not changed. During the second period of the experiment parameters of JG cells' diameters were increased. The volumes of cells (Types I, II, III) and their cytoplasms were naturally growing: I – by 3.23%, II – by 8,53%, and III – by 2.94% compared to the first period of the experiment. Thus, nuclear-cytoplasmic index was decreased by 4.55, 11.76 and 7.14% respectively.

Changes of the taken parameters of white rats kept in another chemically polluted town (Burshtyn) were similar to the above-described ones. Large and small diameters of JG cells were: Type I – (10,84 ± 0,69) and (7,73 ± 0,32) mm, types II and III – (11,29 ± 0,43) and (8,54 ± 0,63) and (12,08 ± 0,81) and (9,19 ± 0,70) mm respectively. The volume of these cells was increased by 19.84, 21.58 and 4.17% respectively compared to the control group. Nuclear-cytoplasmic index of cells (granularity of types I and II) was reduced by 23.81 and 35.29% ($P < 0.05$), and III – in 7,14%. In the kidneys of rats kept in that region for four months the diameters of JG cells continued to grow, but with less intensity. The volumes of JG cells were increased: Type I – by 4.64, II– by 7,28, III – by 3,81% respectively. Nuclear-cytoplasmic index was decreased by 5.00, 6.25 and 7.69% respectively compared to that of animals kept in Burshtyn for two months.

Large and small diameters of JG cells of white rats kept in the region of increased radiation were: type I (10,47 ± 0,21) and (7,58 ± 0,35) mm, II – (10,98 ± 0,52) and (8,36 ± 0,57) mm, III – (11,86 ± 0,45) and (9,12 ± 0,28) mm respectively. Their volumes were increased by 11.25, 13.49 and 3.52% respectively, and nuclear-cytoplasmic index was decreased by 13.04, 21.05 and 7.14%

compared to the control group. Four months later, diameters of JG cells of all types tended to be increased. The volume of these cells compared to the first period of the experiment was increased by 6.45, 9.07 and 2.24% respectively, and cytoplasmic volume got the same changes (see Table 1). Reducing of nuclear-cytoplasmic index was more significant in JG sells (granularity of type II) and was equal to 11.76% compared to that of animals kept in the increased radiological control region for two months.

Morphometric parameters of JG sells of white mice varied depending on the duration of their stay in the study regions, not taking into account the animals kept in positive ecological region (Kosiv), where changes were not determined. (Table 2).

Table 2: Morphometric indices of juxtaglomerular cells of mice kept in different parts of the Carpathians for two (A) and four (B) months, M ± m

Indices, term of experiment		Types of granularity of juxtaglomerulocytes		
		I	II	III
Kosiv				
Volume of a cell, mkm^3	A	129,07±19,82	160,53±13,84	256,29±30,78
	B	126,31±16,98	164,18±18,09	253,15±21,46
Volume of a nucleus, mkm^3	A	36,48±4,32	34,70±14,91	33,98±2,86
	B	34,95±2,69	33,87±16,73	34,05±3,24
Volume of cytoplasm, mkm^3	A	92,77±13,86	129,82±10,97	224,33±23,57
	B	91,90±11,43	127,68±14,26	223,80±19,46
Nuclear-cytoplasmic index s.u.	A	0,42±0,06	0,29±0,03	0,15±0,03
	B	0,41±0,04	0,30±0,05	0,15±0,02
Kalush				
Volume of a cell, mkm^3	A	136,12±21,03	175,80±18,24*	269,33±24,15*
	B	147,03±16,28*	196,25±15,47* ^{o}	283,92±19,06*
Volume of a nucleus, mkm^3	A	38,25±3,97	35,70±2,86	32,71±2,52
	B	37,81±2,35	33,87±3,14	30,96±1,89
Volume of cytoplasm, mkm^3	A	98,23±17,14	141,32±16,71	236,95±22,06
	B	109,67±14,35 ^{o}	165,19±12,98* ^{o}	252,09±15,28*
Nuclear-cytoplasmic index, s.u.	A	0,39±0,05	0,26±0,04*	0,14±0,02
	B	0,35±0,03* ^{o}	0,22±0,03* ^{o}	0,13±0,03
Burshtyn				
Volume of a cell, mkm^3	A	147,95±18,56	179,86±13,72	285,47±21,88
	B	153,08±20,49	198,14±12,03	297,35±30,04
Volume of a nucleus, mkm^3	A	39,78±2,96	36,28±2,21	34,95±3,40
	B	40,90±4,17	38,47±1,89	33,56±2,73
Volume of cytoplasm, mkm^3	A	108,69±14,39	144,56±9,70	250,06±15,48*
	B	113,21±16,75	161,80±8,94* ^{o}	264,13±26,21*
Nuclear-cytoplasmic index, s.u.	A	0,37±0,04*	0,26±0,05*	0,14±0,04
	B	0,36±0,05*	0,23±0,04* ^{o}	0,13±0,03
Stetseva				
Volume of a cell mkm^3	A	137,05±16,49	169,83±11,70	266,75±20,14
	B	142,37±12,98	176,04±15,61	277,29±19,03
Volume of a nucleus, mkm^3	A	36,43±3,58	32,80±5,11	31,65±2,90
	B	37,86±4,02	33,59±2,73	32,98±3,08
Volume of cytoplasm mkm^3	A	100,90±11,27	138,14±10,97	235,42±19,55
	B	105,79±18,31	142,88±15,49	247,30±14,78
Nuclear-cytoplasmic index, s.u.	A	0,37±0,04	0,24±0,04	0,14±0,05
	B	0,36±0,05	0,23±0,03	0,14±0,02

Notes: 1. * – Probability of difference compared to control group ($p < 0,05$).

2. ^{o} – probability of difference between indices of two or four month periods ($p < 0,05$).

In the kidneys of white mice kept in chemically polluted region (Kalush) for two months, large and small diameters of JG cells were: type I – (7,02 ± 0,41) and (5,33 ± 0,19), type II – (7,64 ± 0,61) and (5,78 ± 0,30), type III – (8,69 ± 0,39) and (6,80 ± 0,31) mm respectively. Compared to control group the cells' volumes were increased by 5.38, 9.64 and 5.09%, and nuclear-cytoplasmic index was decreased – by 7.69, 11.54 and 7.14% respectively (Table 2). Four months later the diameters of JG cells (all three types) were slightly increased, as their volumes: Type I – by 8.01%, II – by 11.63% and III – by 5.42% compared to the first period of the experiment. Nuclear-cytoplasmic index of cells (types I, II III) was decreased by 11.43, 18.18 and 7.69% compared to that of animals kept in Kalush for two months.

After being kept for two months in Burshtyn the mice have got increased diameters of JG cells. The indicators of JG cells' volumes were increased: type I – by 11.46, II – by 12,04 and III – by 11.38% and nuclear-cytoplasmic index was decreased by 13.51, 11.54 and 7 14% respectively, compared to control group. In the second period of the experiment, the diameters, volumes of JG cells and their cytoplasm were revealed to be increased. The most significant changes were in JG cells (type II): volume was increased by 10.16%, nuclear-cytoplasmic index was decreased by 13.04%. The majority of JG cells was distributed in the middle membrane of glomerular arteriole near the vascular pole of the renal corpuscle. In contrast to animals kept in positive ecological region, in some areas of the vessels the second row of JG cells were formed. Granular cells were also found in the middle membrane of intraglomerular membrane of arteriole, and occasionally – in the walls of interlobular artery. Their number in this case, were growing to 3-4, 1-2 cells were usually found.

Two month later in white mice kept in region of increased radiological control large diameter of JG cells ranged from (7,02 ± 0,33) to (8,68 ± 0,29) mm, and small one – from (5,30 ± 0,41) to (6,78 ± 0,35) mm. The volume of cells (type I) was increased by 6.18%, II – by 5,79% and III – by 4.18% respectively, and nuclear-cytoplasmic index was decreased by 13.51, 20.83 and 7.14% respectively, compared to control group. During the second term of experiment similar changes took place with the parameters in the kidneys of white mice kept in Stetseva, Snyatyn region.

As for their ultrastructure, JG cells differed from smooth muscle cells because of their cytoplasm containing of polymorphic granules (Fig. 1) endotheliocytes of afferent arteriole, fibroblasts of its adventitia, and in the part of the gat penetrated into the glomerular basement membrane capsule. JG cells' nuclei had different shape and size. Nuclear membrane formed folds and invagination. Chromatin granules localized predominantly in the peripheral part of carioplasma. Cytoplasmic matrix of JG cells is lighter than in smooth muscle cells and contained single polysomes and free ribosomes.

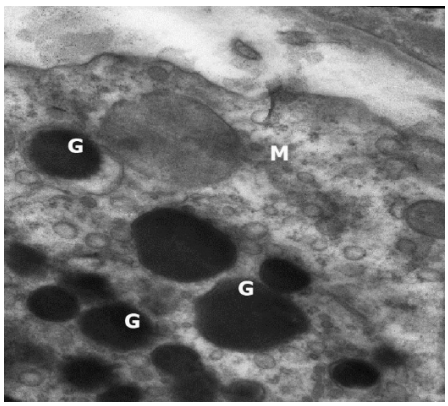

Fig 1: Juxtaglomerular cells of white rats, kept in a chemically polluted town Burshtyn for four months

G – granules of varying degrees of maturity,
M – micropinosytosnic vesicles. Col.: 10 000.
Processes of these cells contacted with

The next stage of work was to study the structure of macula densa cells of animals being under study. In the kidneys of rats after two-month period of being kept in positive ecological region (Kosiv) large and small diameters of their cells were (11,42 ± 0,89) and (8,75 ± 0,64) mm, and mice – (8,89 ± 0,78) and (6,90 ± 0,42) mm respectively. After four months of their keeping

investigated parameters of nuclear cytoplasmic index were increased in the kidneys of white rats by 2,38%, and in mice – by 3.57% compared with that of animals in the first period of the experiment (Table 3).

Table 3: Morphometric indices of macula densa cells of white rats (A) and white mice (B), kept in different parts of the Carpathians

Indices	Period of experiment			
	A		B	
	2 months	4 months	2 months	4 months
Kosiv				
Volume of a cell, mkm^3	599,36±76,81	587,22±93,43	292,65±34,72	281,85±29,67
Volume of a nucleus, mkm^3	177,34±20,11	175,62±18,74	63,46±14,59	64,23±12,85
Volume of cytoplasm, mkm^3	423,94±32,63	412,58±56,31	230,79±29,86	218,39±18,72
Nuclear-cytoplasmic index, s.u.	0,42±0,06	0,43±0,04	0,28±0,04	0,29±0,05
Kalush				
Volume of a cell, mkm^3	628,95±43,71	647,90±75,88*	298,56±34,90	309,84±50,12
Volume of a nucleus, mkm^3	185,37±28,79	183,26±20,45	63,04±12,90	61,89±8,62
Volume of cytoplasm, mkm^3	444,63±39,82	464,59±51,28*	235,41±27,65*	249,73±19,55*
Nuclear-cytoplasmic index, s.u.	0,42±0,04	0,39±0,06*	0,28±0,05	0,26±0,03*
Burshtyn				
Volume of a cell, mkm^3	640,85±79,32	655,12±98,73*	309,67±52,49	317,46±33,12
Volume of a nucleus, mkm^3	182,97±25,68	180,75±31,20	65,82±11,00	63,90±7,12
Volume of cytoplasm, mkm^3	459,26±33,59	476,19±40,28*	243,94±29,85	254,67±38,43*
Nuclear-cytoplasmic index, s.u.	0,40±0,06	0,38±0,05*	0,27±0,04	0,25±0,03*
Stetseva				
Volume of a cell, mkm^3	626,49±89,73	632,00±68,21	293,41±25,32	295,98±33,16
Volume of a nucleus. mkm^3	180,96±24,38	178,32±20,47	62,45±11,06	59,87±6,20
Volume of cytoplasm, mkm^3	447,92±50,43	454,81±42,66	237,08±29,40	240,53±31,57
Nuclear-cytoplasmic index, s.u.	0,41±0,06	0,40±0,07	0,27±0,05	0,26±0,04*

Note. * – Probability of differences compared to control group ($p < 0,05$).

Large and small diameter of macula densa cells in the kidneys of rats and mice after a two–month period of being kept in chemically polluted town (Kalush) were (11,63 ± 0,48) and (8,87 ± 0,55) m and (8,89 ± 0 78) and (6,90 ± 0,42) mm respectively. Cells' volume was increased in rats by 4.94% and in mice – by 2.02%, and nuclear-cytoplasmic index remained unchanged compared to control group. During the second period of the experiment the reduced cells' volume and nuclear-cytoplasmic index of all experimental animals was the same, amounting to 7.69% compared to the first period. All other studied parameters of macula densa cell of white rats and white mice kept in Kalush for four months were characterized by a small increase compared to the animals of the first period of experiment.

In rats kept in Burshtyn for two months the increase of volume of macula densa sells by 6.92%, increase of the volume of the nucleus and cytoplasm by 3.17 and 8.333% respectively and reducing of nuclear-cytoplasmic index by 5.00% compared to control group were noticed. In mice, the volume of cells, their nucleus and cytoplasm were increased by 5.82, 3.72 and 5.71% respectively, and nuclear-cytoplasmic index tended to decrease.

In the second period of the experiment the volume of macula densa cells, their nuclei and cytoplasm of all studied animals were increased slightly compared to the two–month period of their keeping in Burshtyn and nuclear cytoplasmic index was reduced in rats by 5.26% , in mice – by 8,00%.

In the kidneys of all animals kept in Stetseva (Snyatyn region), for two periods, morphometric indices of macula densa were remained unchanged, but nuclear-cytoplasmatic index, was increased respectively by 5.70 and 16.61% (Table 3).

Thus, the taken data may be a manifestation of adaptive changes of renal endocrine

juxtaglomerular complex on man–made pollution effect.

5. Conclusions:

The trend to increasing of diameters and volumes of cell, cytoplasm's volume and reducing of nuclear cytoplasmatic index of juxtaglomerular cells of all types of granularity and macula densa cells of animals kept in bad ecological regions: chemically polluted towns Kalush and Burshtyn, region of increased radiological control – Stetseva, Snyatin region was determined. The most significant changes of cells (granularity of type II) were found. The least sensitive cells to the anthropogenic environmental factors were cells (granularity of type III) and macula densa cells. The dependence of morphometric indices and ultrastructural characteristics of endocrine juxtaglomerular cells and macula densa cells in the kidneys of animals under study on the degree of pollution and the duration of the complex anthropogenic factors of different regions of the Carpathians was proved.

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