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The Phospholipid Composition Changes in White Rats Hepatocyte Chromatin under Conditions of Vagotomy, Solarectomy and Their Combination

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Keywords: *vagotomy, solarectomy, chromatin, phospholipids, phosphoinositides, sphyngomyelins, phosphatidylcholine, phosphoinositidic cycle*

Investigation of molecular-cellular mechanisms of regulation of vegetative nervous system (VNS) adaptation function with the purpose of revealing of organism's various systems functioning have significant importance in the complex of leading problems in modern biomedical science [1,2]. The study of molecular mechanisms of VNS (n. vagus as parasympathic and pl. solaris as sympathetic) peripheral parts action on normal functioning of subcellular structures and cells in whole is of a special interest. From this point of view the investigation of VNS regulatory role in structural organization and functional activity of hepatocytes chromatin becomes very actual.

The purpose of this study was the revealing of qualitative and quantitative changes of hepatocytes chromatin phospholipids (PL) and phosphoinositides (PI) after 7 days of subdiaphragmal bilateral vagotomy (incision of n. vagus), solarectomy (removal of pl. solaris), and their combination.

Experiments were carried out on 70 white male rats. Subdiaphragmal bilateral vagotomy, solarectomy and their combination were performed under light ether anesthesia. Rats were decapitated 7 days after surgical manipulations under anesthesia, too. The nuclei from cells of perfused liver were purified by Blobel and Potter [3]; extraction of chromatin from isolated nuclei was done by Umansky et al [4] and their PL concentration was carried out by Kargapolov [5], protein was

determined by Lowry [6], fractionation of chromatin PL have been organized by Bergelson method of thin-layer chromatography on plates with silicagel "L" [7]. Poly-PI of chromatin were extracted from the residues obtained after the extraction of PL through selective acidic extraction. Fractioning of PI was performed by microthinlayer chromatography on plates with silicagel KCK impregnated with potassium oxalate. The quantitative identification of PL and PI was performed by Ames through their mineralization and subsequent determination of the inorganic phosphorus content in them [8]. The results were treated by Student's method and SPSS 12.0 program.

Table 1

Peculiarities of quantitative changes of phospholipids (mkg of lipid phosphorus/mg protein) in hepatocyte chromatin under the conditions of vagotomy, solarectomy and their combination (absolute values $M \pm m$,% deflection from control, $n = 8$)

Phospholipids	Control	Vagotomy	% defl	Solarectomy	% defl	Combination	% defl
Sphyngomyelins	11.23 ± 0.3	8.80 ± 0.27 $p < 0.001$	-21.6	6.82 ± 0.26 $p < 0.001$	-39.6	5.37 ± 0.27 $p < 0.001$	-52.2
Phosphoinositides	9.38 ± 0.28	11.5 ± 0.2 $p < 0.001$	+22.0	6.7 ± 0.26 $p < 0.001$	-28.6	10.5 ± 0.2 $p < 0.01$	+12.0
Phosphatidylcholines	24.8 ± 0.3	20.7 ± 0.2 $p < 0.001$	-16.5	21.5 ± 0.3 $p < 0.001$	+15.6	17.5 ± 0.21 $p < 0.001$	+14.3
Phosphatidylethanolamines	15.9 ± 0.3	10.5 ± 0.26 $p < 0.001$	-33.8	11.32 ± 0.23 $p < 0.001$	-28.6	10.13 ± 0.2 $p < 0.001$	-36.1
Cardiolipins	4.52 ± 0.24	4.30 ± 0.23	-4.8	3.6 ± 0.23 $p < 0.02$	-20.3	5.36 ± 0.4 $p < 0.02$	+18.5
Phosphatidic acids	4.82 ± 0.21	4.35 ± 0.24	-9.0	4.01 ± 0.21 $p < 0.001$	-16.8	4.93 ± 0.21	+2.3
Sum of neutral phospholipids (SNPL)	51.9 ± 0.3	40.0 ± 0.7 $p < 0.001$	-23.0	39.7 ± 0.6 $p < 0.001$	-23.7	33.0 ± 0.5 $p < 0.001$	-36.4
Sum of acidic phospholipids (SAPL)	18.7 ± 0.4	20.1 ± 0.6 $p < 0.1$	+7.4	14.3 ± 0.5 $p < 0.001$	-23.6	20.8 ± 0.5 $p < 0.01$	+11.1
Total phospholipids	70.7 ± 0.7	60.1 ± 1.1 $p < 0.001$	-15.0	53.9 ± 1.09 $p < 0.001$	-23.7	53.8 ± 0.9 $p < 0.001$	-23.8
SNPL / SAPL	2.8 ± 0.1	1.99 ± 0.04 $p < 0.001$	-28.6	2.79 ± 0.1	0.0	1.59 ± 0.03 $p < 0.001$	-43.01

The results obtained (Table 1) testify the reliable decrease of total PL of hepatocytes chromatin in conditions of vagotomy approximately by 15%, solarectomy

– 23.7%, and their combination - 23.8%. This highlights the possible depression of chromatin matrix activity through the changes in hydrophobic interactions between DNA and histones, as well as influence on conformation of proteins and protein-protein interactions with the decrease of accessibility of RNA-polymerases to the promoter region of gene [9,10].

The decrease of absolute and relative contents of sphyngomyelins (SM) is conditioned by depression of chromatin matrix activity, RNA-polymerases I and II, and changes of quantitative correlation between free and bonded forms of enzyme in all cases mentioned [11]. The decrease in content of phosphatidylcholines (PC) and phosphatidylethanolamines (PE) in chromatin of hepatocytes at extreme conditions is of special interest, which bears multidirected character and is more highlighted in the cases of combination of vagotomy and solarectomy. In this respect the disorders in the functional activity of PC cycle and processes of signal transduction [12,13], have the special significance.

From the other hand it is very important to notice the changes in contents of cardiolipins (CL) and phosphatydic acids (PA) resulting the changes in the ratio of neutral and acidic PL, which is more expressed in conditions of vagotomy (decrease by 28.6 %) and its combination with solarectomy (decrease by 43.01%).

The changes in the content of total and individual PL testify the disturbances in structural organization and functional activity of hepatocytes chromatin [1,9]. In this case their own role might have the changes in contents of PI, particularly decrease by 28.6% at solarectomy, increase by 22.0% at vagotomy, and increase by 12.0% at their combination. This might be related to the compensatory perturbation in hepatocytes chromatin.

The investigation of qualitative and quantitative changes of PI have shown the following (Table 2).

The decrease of tri-PI is more expressed at solarectomy on the background of relatively stable concentration of di-PI. The quantitative displacements in the content of mono-PI are more expressed. While their concentration is increased in the conditions of vagotomy and its combination with solarectomy (approximately 53.6% and 43.5% relatively), in conditions of solarectomy we have determined decrease of their content approximately by 37%.

These data testify serious disturbances in the functioning of PI cycle and signal transduction [13,14]. The proof of noticed is the changes in the ratios of individual PI bearing different character and more expressed in conditions of solarectomy (Table 3). The data given in the table 3 figure out possible disturbances of enzymatic systems regulating PI metabolism, particularly tri-PI/di-PI (decrease by 40.1% at solarectomy), di-PI/mono-PI (decrease by 52.1% at solarectomy), tri-PI/mono-PI (decrease by 41.5% at vagotomy, and 49.2% at its combination with

solarectomy).

Table 2

Changes in content of mono- and poly-Phosphoinosotides (mkg of lipid phosphorus/mg.protein) in hepatocytes chromatin under the conditions of vagotomy, solarectomy, and their combination (absolute values $M \pm m$,% deflection by control, $n = 8$)

PI	Control	Vagotomy	% defl	Solarectomy	% defl	Combination	% defl
Mono-PI (3)P	3.97 ± 0.35	6.10 ± 0.03 $p < 0.001$	-53.6	2.50 ± 0.02 $p < 0.001$	-37.0	5.70 ± 0.02 $p < 0.001$	+43.5
Di-PI $(3,5)P_2$	2.83 ± 0.02	3.00 ± 0.7 $p < 0.05$	+6.0	2.75 ± 0.02 $p < 0.02$	-4.1	2.90 ± 0.02	+2.4
Tri-PI $(3,4,5)P_3$	2.57 ± 0.02	2.35 ± 0.0036 $p < 0.001$	-8.56	1.48 ± 0.02 $p < 0.001$	-42.41	1.91 ± 0.007 $p < 0.001$	-25.6
Sum of PI	9.2 ± 0.17	11.49 ± 0.07 $p < 0.001$	+24.9	6.71 ± 0.13 $p < 0.001$	-27.1	10.5 ± 0.04 $p < 0.001$	+13.9

Summarizing all data obtained, we can state about the deep displacements in the structural organization and functional activity of genetic apparatus of liver cells at investigated extreme states of organism.

Table 3

Changes of ratios of individual Phosphoinositides in conditions of vagotomy, solarectomy, and their combination ($M \pm m$,% deflection by control, $n = 8$)

Ratio	Control	Vagotomy	% defl	Solarectomy	% defl	Combination	% defl
Tri-PI/ di-PI	0.91 ± 0.008	0.78 ± 0.017 $p < 0.001$	-13.3	0.545 ± 0.008 $p < 0.001$	-40.1	0.65 ± 0.03 $p < 0.001$	-28.5
Tri-PI/ mono-PI	0.65 ± 0.007	0.38 ± 0.003 $p < 0.001$	-41.5	0.59 ± 0.01 $p < 0.001$	-9.2	0.33 ± 0.002 $p < 0.001$	-49.2
Di-PI/ mono-PI	0.71 ± 0.01	0.49 ± 0.01 $p < 0.001$	-30.9	1.08 ± 0.01 $p < 0.001$	+52.1	0.51 ± 0.003 $p < 0.001$	-28.1

Thus, the results obtained once more verify the reality of Orbeli's biological conceptions about the adaptational-trophical action of peripheral parts of VNS on subcellular structures, cells, organs and organism in whole[15].

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The Phospholipid Composition Changes in White Rats Hepatocyte Chromatin under Conditions of Vagotomy, Solarectomy and Their Combination

There are shown the changes in the qualitative and quantitative composition of phospholipids, including phosphoinositides, in white rats hepatocyte chromatin under conditions of vagotomy, solarectomy and their combination, testifying serious disturbances in structural organization and functional activity, also in the functioning of phophoinositidic cycle of signal transduction of hepatocyte chromatin.

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Изменения состава фосфолипидов хроматина гепатоцитов белых крыс в условиях ваготомии, солярэктомии и их сочетания

Показаны изменения качественного и количественного состава фосфолипидов (в том числе фосфоинозитидов) хроматина гепатоцитов белых крыс в условиях ваготомии, солярэктомии и их сочетания, свидетельствующие о серьезных расстройствах в структурной организации и функциональной активности, а также функционировании фосфоинозитидного цикла сигнальной трансдукции хроматина гепатоцитов.

Ակադեմիկոս Կ. Գ. Ղարագյոզյան, Գ. Ա.Հովեյան, Գ. Ա. Գևորգյան, Ա. Գ. Հովեյան

Սպիտակ առնետների հեպատոցիտների քրոմատինի ֆոսֆոլիպիդների բաղադրության փոփոխությունները վագոտոմիայի, սոլարէկտոմիայի եւ դրանց զուգակցման պայմաններում

Յույց են տրված սպիտակ առնետների հեպատոցիտների քրոմատինի ֆոսֆոլիպիդների ներառյալ ֆոսֆոինոզիտիդների որակական եւ քանակական կազմի փոփոխությունները վագոտոմիայի, սոլարէկտոմիայի եւ դրանց զուգակցման պայմաններում՝ վկայելով հեպատոցիտների քրոմատինի կառուցվածքային կազմակերպման եւ ֆունկցիոնալ ակտիվության, ինչպես նաեւ ազդանշանային տրանսդուկցիայի ֆոսֆոինոզիտիդային օղակի լուրջ տեղաշարժերի մասին:

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