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LIPIDS OF CARDIOMYOCYTES MEMBRANES STRUCTURES IN RATS AT ARTIFICIAL HYPOBIOSIS

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Abstract

There was studied an influence of artificial hypobiosis in the conditions of hypoxia-hypercarnia in rats on the quantity of neutral lipids, phospholipids and its fatty acids in membranes structures of cardiomyocytes (microsomes and inner membranes of mitochondria). The received results – the content of lipids, individual phospholipids, cholesterol testify to the modification of lipid component of cardiomyocytes mitochondria inner membrane (less the microsomal fraction) that characterizes modulation of cellular membranes structural-functional state. There was noticed the possibility of attraction of membrane phospholipids (sphingomyelin, phosphatidylserine and phosphatidylinositol) to the signal ways activation at hypobiosis. There was revealed redistribution of fatty acids of mitochondria inner membrane at hypobiosis that leads to increase of the level of unsaturated fatty acids. There was noticed the possibility of participation of monoenic unsaturated fatty acids in protection of cellular structures from oxidizing stress and increase of the content of arachidonic and docosahexaenoic acids – precursors of biologically active substances – can be connected with its attraction to regulatory systems at hypobiosis. There is presupposed that the state of artificial hypobiosis is characterized with stress-reaction that leads to optimal reconstruction of lipid and fatty acid content of membrane lipids directed on support of cardiomyocytes functional activity.

Keywords: lipids, fatty acids, membranes, mitochondria, microsome, cardiomyocytes, artificial hypobiosis.

1. Introduction

Theoretical and practical importance of the hypobiosis problem [1] with paying attention to the current studies favored the grounding of new direction in biological science – hypobiology that investigates the specific state of living organisms that takes place at medium factors action and caused by decrease of functional activity [2].

Membrane lipids play an important role at transfer of mammal organism to the decreased level of functional activity in conditions of the low temperature of environment [3, 4]. In particular the modification of fatty acid content influence the intensity of several metabolic process-

es and acts as compensatory mechanism that provides membranes functional possibilities at changed conditions [5].

The lipids participation in change of membrane microviscosity is an important aspect of lipids role in organism adaptation to environment extreme factors. The idea of homeoviscous adaptation of organism to the low temperature effect is well-known [6]. The increase of part of phospholipids unsaturated fatty acids, increase of phosphatidylethanolamine quantity and decrease of cholesterol/phospholipids ratio are considered as key factors of homeoviscous adaptation.

The chemical content of phospholipids that are the structural components of cellular membranes plays the leading role in its functioning and course of different processes in cells. Especially saturated fatty acids (SFA) are the main energetic substance for cardiomyocytes. Owing to ability to increase unsaturation of phospholipids acyl chains, decrease microviscosity of cellular membranes unsaturated fatty acids (USFA) act as the important factor of regulation of membrane permeability and membrane linking proteins functioning [4].

The main provider of energy accumulated in ATP in eukaryotic cells is mitochondria which functional activity is provided first of all by the inner membrane that contains all components necessary for ATP synthesis that is determinative factor for cell's execution of specific functions including formation of response on external influence [7].

Aim of the work is the study of influence of artificial hypobiotic state on quantitative content of general lipids, cholesterol, phospholipids and fatty acids in cellular organelles with the different functions – mitochondrial and microsomal fractions of cardiomyocytes in rats.

2. Materials and methods

In experiments were used 42 white not purebred rat-males with weight 180–200 g. Experiments were carried out according to requirements of "European convention on protection of spinal animals used with experimental and other scientific aim" (Strasbourg, 1986).

Animals were divided into 2 groups: 1 – the control one (intact animals); 2 – rats that were modeled the state of artificial hypobiosis. This state was created by methods of Bakhmet'ev-Giaja-Anjus combining hypoxia-hypercapnic influence with external cooling as described in details [8]. For this aim animals were placed in hermetically closed chamber with volume 3 dm^3 at external temperature 3–4 ºC. During 3–3,5 hours stay in chamber in animals was developed hypobiotic state at which body temperature decreased to 16 ºC. Animals underwent decollation in the state of normothermia (body t=37 ºC) and at artificial hypobiosis (body t=16 ºC).

By the method of differential centrifugation was received microsomal (MM) fraction of cardiomyocytes and mitochondrial fraction from which was received the inner membrane (IM) of mitochondria [9]. Lipids were extracted by Folch method [10]. Phospholipids were divided by the method of thin-layer chromatography (TLC), the quantity of individual phospholipids (PL) and cholesterol (CS) was detected [11].

Hydrolysis and methylation of FA lipids was realized using method presented in the work [12]. Gas chromatographic analysis of FA methyl ethers was carried out on gas chromatograph Trace GC Ultra (USA) with flame-ionization detector. The conditions of experiment: geyser temperature 140–240 ºC, detector temperature 260 ºC, analysis duration 65 min. Fatty acids (FA) were identified using standard model Supelco 37 Component FAME Mix. Quantitative assessment of FA spectrum was carried out using method of rationing planes of methylated derivative FA peaks and its content was detected in percentage from the summary content of all FA.

Statistic processing of experimental data was carried out using common methods of variation statistics. The probability of indices difference was assessed on Student t-criterion. Differences between compared indices were considered as veritable at significance level $P<0,05$.

3. Results of research and discussion

The stay of animals in hypoxia-hypercarpnic medium at lowering of body temperature (artificial hypobiosis) results in increase of content of IM mitochondria lipids: general lipids in

average by 24,7 %, general PL by 24,2 %, CS by 40,7 % comparing with control. The value of molar CS/PL ratio that characterizes modification of membrane structural-functional state increases by 16 % (**Table 1**).

Table 1

Lipids content (mcg/mg of protein) in mitochondria inner membrane and microsomal fraction of cardiomyocytes in rats at artificial hypobiosis (M±m, n=8)

Lipids	Mitochondria inner membrane		Microsomal fraction	
	Control	Hypobiosis	Control	Hypobiosis
General lipids	271,6±21,0	338,4±30,2*	298,6±21,0	333,4±22,2
Cholesterol	2,7±0,1	3,8±0,2*	7,7±0,6	8,8±0,5*
General phospholipids	208,2±18,1	258,5±21,0*	231,2±18,1	255,5±21,0
Phosphatidylcholine	80,1±6,8	98,7±7,8*	101,1±5,8	110,7±6,8
Posphatidylethanolamine	57,2±4,1	70,3±6,1*	89,5±4,1	100,3±6,1
Sphingomyelin	9,4±0,7	14,3±1,0*	13,4±0,7	29,3±1,0*
Phasphatidylserine + Phosphatidylinositol	25,2±2,0	43,2±3,1*	27,2±2,0	38,2±3,1*
Cardiolipin	36,2±2,8	30,8±2,3*	–	–
CS/PL	0,025	0,029	0,065	0,060
CS/PC	0,068	0,057*	0,153	0,151
PC/SPM	5,80	4,69*	6,73	3,26*

*Note: molar ratios: CS/PC – cholesterol to phosphatidylcholine; CS/PL – cholesterol to phospholipids; PC/SPM – phosphatidylcholine to sphingomyelin; * – P<0,05 comparing with control*

Content of individual PL of mitochondrial membrane – phosphatidylcholine (PC) and phosphatidylethanolamine (PEA) increases in average by 23,2 % and 22,9 % respectively comparing with control (**Table 1**). The content of minor PL increases more essentially at hypobiosis especially sphingomyelin (SPM) and phosphatidylserine + phosphatidylinositol (PS+PI) increases by 52,1 % and 71,4 % respectively comparing with control. At the same time the content of cardiolipin in IM decreases in average by 14,9 % that is connected with modification of functional activity of cardiomyocytes mitochondria IM at artificial hypobiosis [8]. There were not revealed any changes of PL lizoforms content in cardiomyocytes IM in conditions of experiment that testifies to the absence of oxidative processes activation at hypobiosis.

For microsomal fraction (MM) of cardiomyocytes at artificial hypobiosis in rats were observed the less intense changes in lipids content (**Table 1**). Increase of PL content by 11 % and CS – by 14,3 % comparing with control does not results in changes of CS/PL ratio. At the same time there was observed an increase of minor PL content especially: SPM in average in 2,2 times, PC+PI – by 40,4 % comparing with control. There were not revealed any changes in cardiomyocytes MM PL lizoforms content in the conditions of experiment (**Table 1**).

So the stay of animals in hypoxia-hypercapnic medium at lowering body temperature causes reconstruction of lipid component of mitochondria IM and also somewhat less intense changes in the content of cardiomyocytes microsomal fraction lipids.

Taking into account the received results there was further detected the FA lipids content of mitochondria IM using method of highly sensitive gas chromatography. There were identified the next FA: lauric C12:0, myristic C14:0, pentadecanoic C15:0, palmitic C16:0, palmitoleic C16:1, margarine C17:0, hexadecenoic C17:1, stearic C18:0, oleic C18:1, linoleic C18:2, linolenic C18:3, gadoleic C20:1, eicosatrienoic C20:2, arachidonic C20:4, eicosenic C21:0, docosahexaenoic C22:6.

In the control saturated fatty acids (SFA) mainly presented by palmitic and stearic acids and unsaturated fatty acids (USFA) are divided into monoenic (especially oleic and palmitoleic) and polyenic ones (linoleic, arachidonic and docosahexaenoic). The summary level of USFA is higher than SFA (saturation coefficient is 0,74) **(Fig. 1)**.

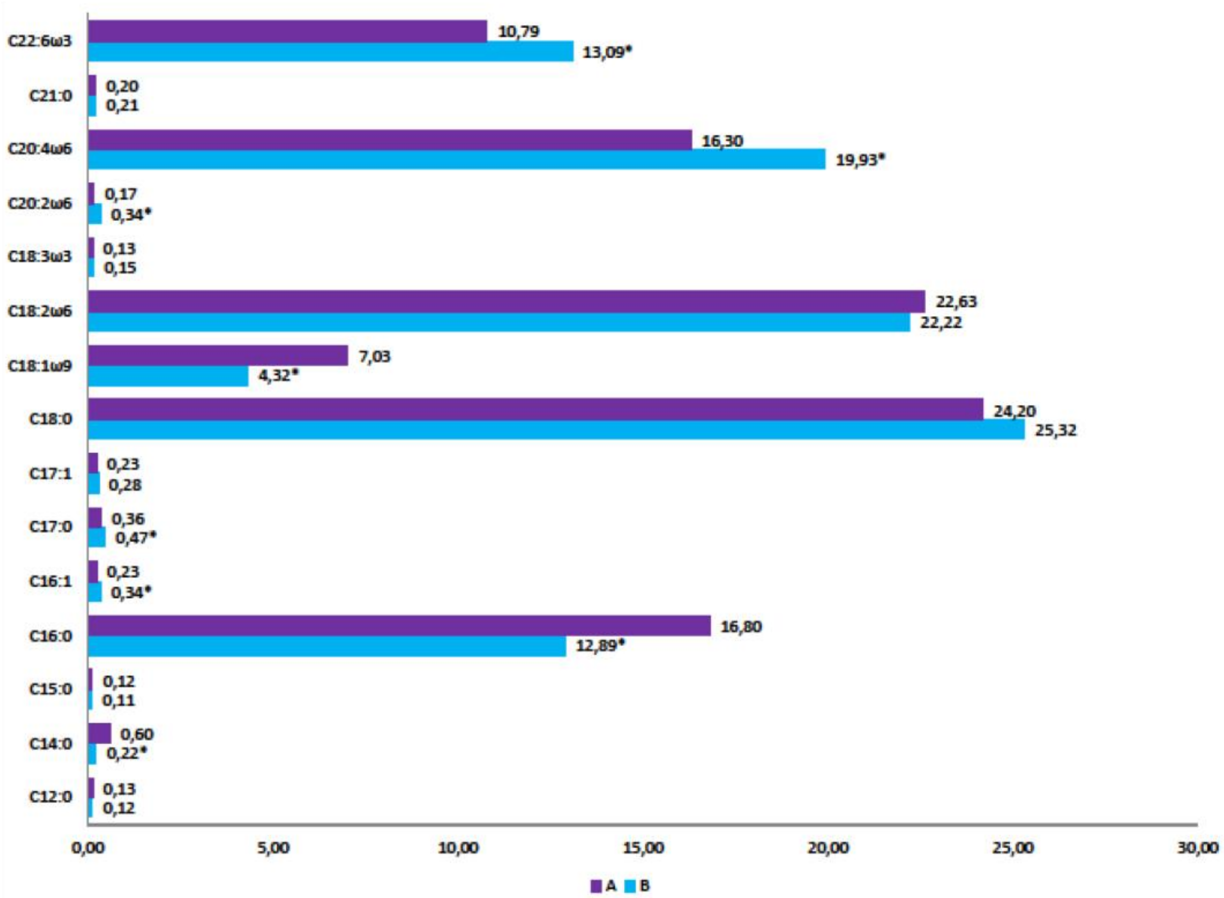

Fig. 1. Fatty acid content of lipids of cardiomyocytes mitochondria inner membrane in rats at artificial hypobiosis (mass fractions of fatty acids in % from the sum of fatty acids; n=6, n – animals number in group): A – control group; B – research group (artificial hypobiosis); * – $P<0,05$ comparing with control.

Animals stay at artificial hypobiosis results in many-sided redistribution of FA content, saturation coefficient is 0,65 (**Fig. 1**). The summary SFA content decreases especially palmitic one by 23,3 % comparing with control. It can explain for example the deficiency of energetic substance in cells at hypobiosis. The summary USFA increases and the content of monoenic ones probably decreases (4,93±0,11 against 7,47±0,22 % in control). Moreover the oleic acid content decreases by 39 %, and palmitoleic one increases by 48 % comparing with control ($P<0,05$). The detected changes are probably connected with protection of cellular membrane from oxidative processes activation because there were not revealed any reliable changes in content of irreplaceable FA – linoleic and linolenic ones that are more often oxidized. It is known an effect of decrease of intensity LPO processes by monoenic SFA at the expense of reactive oxygen species (ROS) neutralization [13]. Besides that oleic acid is characterized as endogenic interceptor of ROS (as the result its pool can be decreased) and palmitoleic acid acts as cytoprotector [14].

The summary content of polyenic USFA increases (55,73±0,42 against 50,12±0,31 % in control) mainly at the expense of increase of content of such functionally significant polyenic USFA as arachidonic and docosahexaenoic acids by 22,1 % and 21,3 % respectively comparing

with control (**Fig. 1**). Arachidonic acid as a component of phospholipids of cellular membranes interacts with protein complexes having influence on functioning of receptors, transport and signal systems [15]. One of mechanisms of docosahexaenoic acid action is also connected with modification of fatty acid phospholipid content of cellular membranes that influences ionic channels and membrane receptors [16].

At analysis of received results we must take into account that lipids are the structural components of biomembranes that condition its physical-chemical and functional properties and also are included to the content of cells signal systems [17]. For cardiomyocytes mitochondria IM is typical the increase of general lipids, CS, PL and individual PL, changes of values of molar ratio CS/PL, CS/PC and PC/SPM that indicates the adaptive modification of membrane structural-functional state at artificial hypobiosis [18]. As to the role of membrane CS there are data that testify to the increase of CS content, aggregation of membrane linking proteins without microviscosity changes so the CS participation in membrane lipids metabolism is possible [19].

The study of fatty acid spectrum of mitochondria IM lipids testify to the FA redistribution, increase of USFA level and decrease of SFA at hypobiosis that can testify to adaptation of membranes physical-chemical properties. The possibility of attraction of monoenic USFA to protection from oxidative stress at hypobiosis is very important.

Considering the direct participation of USFA in regulation of most cellular processes [20] the increased content of arachidonic and docosahexaenoic acids that are precursors of biologically active substances testifies to the attraction of mitochondria IM FA to the cellular regulation of artificial hypobiosis state.

On the contrary for microsomal fraction the changes in lipids content are less intense excepting the content of minor PL (SPM and PC+PI sum) that testifies to signal ways activation at hypobiosis.

So the cardiomyocytes organelles lipids take part in modification of membrane structures functions at hypobiosis, especially adaptation of energetic systems to hypobiosis conditions is connected with change of content of mitochondria IM cardiolipin as it was noticed above.

4. Conclusions

The animals stay in hypoxia-hypercapnic medium at lowering of body temperature (artificial hypobiosis) causes specific changes of lipid content of mitochondria inner membrane and microsomal fraction that testifies to modulation of structural-functional state of cardiomyocytes cellular membranes that can condition the adaptation of heart cells to artificial hypobiosis state.

There was demonstrated the possibility of regulatory role of cellular membranes phospholipids at action of hypobiosis factors (hypoxia, hypercapnia and hyperthermia) and signal ways activation including participation of sphingomyelin, phosphatidylinositol and phosphatidylserine.

The revealed redistribution of fatty acids of mitochondria inner membrane is connected with adaptation of structural order of lipid component and the changes of content of oleic, arachidonic and docosahexaenoic acids indicate its attraction to the regulatory systems at hypobiosis.

There is presupposed that the state of artificial hypobiosis is characterized with stress-reaction that results in optimal reconstruction of lipid and fatty acid content of membrane lipids directed on support of cardiomyocytes functional activity.

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