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The protective action of alpha-tocopherol on the white matter lipids during moderate hypoxia in rats

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Abstract

Hypoxia and ischemia acting on the brain cause alterations of the level of lipids and sterols.
Famile 3.0-3.5-month-old rats were used for the experiment. They were given alpha-tocopherol in the dose of 11.43 mg/kg of body weight through seven days, then underwent hypoxia (7% of oxygen in the breathing mixture) and myelin was isolated in four times after experiment: 4, 24 hours, 14 days and 2 months after experiment.
Three lipids groups were isolated that are neutral lipids, galactolipids and phospholipids. They were quantitatively analyzed with spectrophotocolorimetry. The obtained results indicate that vitamin E administration to animals does not cause significant changes of brain lipids levels. However, alpha-tocopherol administred before moderate hypoxia balances the concentrations of lisophosphatidylcholine and phosphatidylinositide and cerebrosides with control level 2 months after experiment. Vitamin E changes in concentration of the myelin neutral lipids.
Vitamin E administered before experimental moderate hypoxia stabilizes some membrane lipids and could be used in brain hypoxia.

Key words: *vitamin E, lipids, myelin, moderate hypoxia, rats*

Introduction

In hypoxia and ischemic conditions of the brain many changes in contents and lipids metabolism [23,24], membrane sterols [1,6,25] occur and also free oxygen radicals (FOR) are produced.

FOR cause damage of polyunsaturated fatty acids, which belong to the membrane phospholipids [3], and in this way alter several cellular membranes features, mainly ions, water and glucose permability, fluelity and processes connecting with intracellular signals conduction. FOR level, collecting in the organism is due to efficacy of protective skills, that is antioxidants and antioxidative enzymes [11].

Vitamin E (alpha-tocopherol) is one of the natural antioxidants occuring both in humans and animals. Alpha-tocopherol is thought to be an integral compound of the cellular membrane [14,26]

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Table I. Myelin neutral lipids after moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	4 hours	24 hours	14 days	2 months
		after hypoxia			
cholesterol	25.71±1.99	18.49±0.84**	19.49±0.84**	21.66±1.85**	22.94±1.90*
cholesterol esters	0.42±0.04	0.84±0.05**	0.93±0.11**	0.97±0.10**	0.98±0.13**

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
*Statisticcally significant values at **p<0.01 and at *p<0.05*

Table II. Myelin galactolipids after moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	4 hours	24 hours	14 days	2 months
		after hypoxia			
cerebrosides	8.85±1.29	8.42±0.70	8.09±1.17	8.17±0.99	8.21±1.33
sulfatides	3.14±0.60	2.85±0.55	2.37±0.70*	2.56±0.83	2.73±0.57
total galactolipids	12.35±0.69	11.27±0.95*	10.46±1.56**	10.74±1.60*	10.94±1.65*

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
*Statisticcally significant values at **p<0.01 and at *p<0.05*

protecting mainly polyunsaturated fatty acids of phospholipids from oxidation [18].

Except antioxidant acivity, vitamin E influences also cellular answer in hypoxia conditions, because it modulates signal transduction [14] and controls stability and permeability of the membrane [11], it regulates immunological response of the organism, both humoral and cellular [15].

It is thought that vitamin E belongs to the most effective antioxidants. Its antioxidative action is due to the uptake of the molecules initiating autooxydative processes. It decreases FOR level, diminishes lipid superoxides concentration [2,22] and protects nitric oxide accumulation.

It is known that vitamin E through reaction with FOR protects neurons from apoptosis caused by oxidative stress [21] both in hypoxia processes and other central nervous system diseases such as Alzheimer's disease, neoplasms, traumas and aging processes.

From the literature we know [5,9] that vitamin E administration with alpha-lipoic acid together with Methylprednisolone magnify its positive antioxidant action and it might be used in brain hypoxia in humans.

Meydani [14] showed that in experimental ischemia in rats vitamin E dicreases the field of the ischemia and membrane damage. Administration of vitamin E before experimental moderate hypoxia might diminish results of restricted accessible oxygen and protect membrane lipids from changes within cellular membrane mainly myelin yield, structure very sensible to hypoxia.

Material and methods

Femile 3.0-3.5-month-old Wistar rats were used for these experiments. The animals were fed a standard diet and water ad libitum, and kept in controlled conditions at 21°C.

The experimental animals were given vitamin E in the dose of 11.43 mg/kg of the body weight per seven days, then underwent mild hypoxia and brain lipids were analyzed in 4 times: 4 and 24 hours, 14 days and 2 months after experimental moderate hypoxia.

The rats underwent moderate hypoxia by placing them in the chamber containing 7.0% oxygen, 92.9% nitrogen and 0.1% carbon dioxide. The duration of hypoxia was 30 min. Immediately after hypoxia, the

Table III. Myelin phospholipids after moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	4 hours	24 hours	14 days	2 months
		after hypoxia			
Sphingomyelin SM	1.35±0.11	1.56±0.15**	1.87±0.27**	1.67±0.23**	1.46±0.26
Phosphatidylcholine PC	6.46±0.91	6.74±0.79	8.20±1.26**	7.55±0.96*	6.31±0.86
Lysophosphatidylcholine LPC	0.35±0.07	0.56±0.18*	1.41±0.26**	0.75±0.15**	0.48±0.15*
Phosphatidylserine PS	2.70±0.52	2.83±0.38	2.93±0.44	3.20±0.70	2.68±0.46
Phosphatidylinositide PI	0.42±0.04	0.46±0.05*	0.46±0.12	0.69±0.15**	0.50±0.12
Phosphatidylethanolamine PE	11.09±2.12	11.76±1.58	12.99±1.67*	11.78±1.15	11.24±1.69
Plasmalogen PE PPE	8.89±1.12	8.09±1.32*	7.64±0.78*	7.52±1.50*	7.62±1.08**
Plasmalogen PC PPC	3.12±0.55	2.73±0.26	2.51±0.35*	2.46±0.59*	2.69±0.29*
Total phospholipids	34.39±3.80	34.71±3.17	38.11±3.44*	35.68±3.45	32.97±2.14

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
*Statisticcally significant values at **p<0.01 and at *p<0.05*

arteriovenous blood was withdrawn from the tail, and pO_2, pCO_2 and pH were measured using a bloog gas analyzer (Mokro Astrup BMS-2, Radiometer, Denmark). The animals were sacrificed with halothane anesthesia, decapited, and the brains immediately placed in liquid nitrogen.

Myelin was isolated from the brain of control and experimental rats by centrifugation in discontinuous saccharose gradient according to Norton and Poduslo [17]. Myelin fraction lipids were extracted according to Folch et al. [8]. The lipid extract was separated into three groups: neutral lipids, galactolipids and phospholipids on a Florisil column (100-200 mesh, Fluka).

Neutral lipids were eluted with hexane: ethyl ether mixture 8:2 (v/v) according to Kishimoto et al. [12].

Cholesterol and its esters concentrations were estimated after previous chromatographic analysis (one-dimensional thin-layer chromatography) by spectrophotocolorimetric method with ferric chloride.

Galactolipids were eluted from Florisil column with chloroform: methanol mixture 2:1 (v/v) according to Svennerholm method [20].

To distinguish galactolipid fractions into cerebrosides and sulfatides one-dimensional thin-layer chromatography was used and isolated lipids were estimated with spectrophotocolorimetry with orcinole.

Phospholipids isolated with two-dimensional thin-layer chromatography [16] and then evaluated by spectrophotocolorimetry with Fiske-Subarow solution and ninhydrine solution by plasmalogens evaluation.

Statistic procedures

The obtained results were statistically evaluated by nonparametric Mann-Whitney test for independent variables (GraphPad Softwere, Statgraph, USA).

Results

The analysis of parameters of gas and acid-base balance are presented in previous study by Dorszewska and Adamczewska-Goncerzewicz [6].

Tables 1, 2, 3 demonstrate levels of myelin membrane: neutral lipids, galactolipids and phospholipids respectively in controls and in four periods after the experimental hypoxia: 4, 24 hours (early periods after hypoxia), 14 days and 2 months (late periods after hypoxia).

In the class of the neutral lipids (Table 1) estimated statistically significant decrease in cholesterol level in all analyzed periods after the experiment (Mann-Whitney test, $p<0.01$, 4, 24 hours and 14 days and $p<0.05$, 2 months after the experiment) and statistically significant increase of cholesterol esters concentration, remain even to late

Table IV. Neutral myelin lipids after vitamin E administration before moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	Control vit. E	4 hours	24 hours	14 days	2 months
			after hypoxia			
Cholesterol	25.71±1.99	25.72±1.73	24.09±1.69*	22.63±1.48**	23.92±1.38*	25.31±1.52
Cholesterol esters	0.42±0.04	0.43±0.04	0.54±0.06	0.61±0.08**	0.66±0.09**	0.69±0.08**

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
*Statisticcally significant values at **p<0.01 and at *p<0.05*

Table V. Myelin galactolipids after vitamin E administration before moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	Control vit. E	4 hours	24 hours	14 days	2 months
			after hypoxia			
Cerebrosides	8.85±1.29	8.84±1.09	9.09±0.92	8.77±1.34	8.64±1.09	8.86±0.92
Sulfatides	3.14±0.60	3.20±0.37	3.08±0.70	2.92±0.86	2.88±0.57	2.86±0.38
Total galactolipids	12.35±0.69	12.05±1.24	12.17±0.96	11.69±1.98	11.52±1.16	11.74±1.10

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
Any statistically significant differences

periods after experiment (Mann-Whitney test, p<0.01 in all periods after experiment).

Within galactolopids (Table 2) both cerebrosides and sulfatides decreased their levels in comparison with controls in all periods after experment (Mann-Whitney test, p<0.05, sulfatides 24 hours after experimental hypoxia).

On the contrary, in phospholipid class (Table 3) it was shown that sphingomyelin (SM) (Mann-Whitney test, p<0.01, 4, 24 hours, 14 days after the experiment) and lisophosphadidylcholine (LPC) (Mann-Whitney test, p<0.01, 24 hours, 14 days and p<0.05, 4 hours and 2 months after hypoxia) and phosphatidylinositide (PI) (Mann-Whitney test, p<0.01, 14 days and p<0.05, 4 hours after the experiment) together with phosphatidylethanolamine (PE) (Mann-Whitney, test p<0.05, 24 hours after hypoxia) showed increased concentration in all periods after the experiment.

Simultaneously, it was noticed that vitamin E administration (Tables 4-6) to the experimental animals did not cause any significant changes in the content of the meylin lipids. However, alpha-tocopherol, administered before mild hypoxia influenced the level of the analyzed brain sterols (Table 4) and led to only slight level decrease of free cholesterol and diminished its estrification.

In the galactolipids class, vitamin E administered before the experiment influenced the level of these lipids to a smaller extent (Table 5) and did not cause any increase in sulfatides level in all periods after the experiment and cerebrosides level 24 hours and 14 days after hypoxia (it balanced the concentrations of the cerebrozides with control level 2 months after the experiment).

At the same time, vitamin E influenced in the different ways the phospholipids level. Its administration before the experiment practically did not change the SM and PE concentrations (Table 6). In the remaining analyzed phospholipids fractions, mainly within lisocompounds (LPC) and PI much lower increase in the late period after hypoxia (2 months) was observed, practically comming back to the control values. Simultaneously, vitamin E administration caused the increased concentration lasting even 2 months after the experiment and lack of significant influence on the plasmalogens level in hypoxyted animal brain both PE and PC.

Discussion

The previous Wender et al. work [23] and the performed studies indicate that experimental

Table VI. Myelin phospholipids after vitamin E administration before moderate hypoxia (mmol/100 g of myelin)

Lipids	Control	Control vit. E	4 hours	24 hours	14 days	2 months
					after hypoxia	
Sphingomyelin SM	1.35±0.11	1.40±0.14	1.60±0.26*	1.90±0.25**	1.72±0.22**	1.57±0.19**
Phosphatidylcholine PC	6.46±0.91	6.33±0.70	6.67±1.32	8.29±0.93**	8.02±0.98*	6.94±0.93
Lysophosphatidylcholine LPC	0.35±0.07	0.34±0.06	0.55±0.18**	1.40±0.23**	0.85±0.13**	0.38±0.08
Phosphatidylserine PS	2.70±0.52	2.60±0.26	2.88±0.48	2.96±0.40	3.13±0.50	2.65±0.32
Phosphatidylinositide PI	0.42±0.04	0.40±0.05	0.47±0.09	0.53±0.13*	0.69±0.15**	0.43±0.08
Phosphatidylethanolamine PE	11.09±2.12	10.90±1.54	11.64±1.81	12.55±1.63	11.85±1.74	11.44±1.79
Plasmalogen PE PPE	8.89±1.12	8.91±0.78	8.29±1.65	7.83±1.21*	7.49±1.11*	7.44±0.95**
Plasmalogen PC PPC	3.12±0.55	3.15±0.40	2.78±0.57	2.49±0.56*	2.40±0.64*	2.85±0.47
Total phospholipids	34.39±3.80	34.15±2.26	34.88±4.24	37.93±1.90*	36.14±1.86	33.70±2.70

Means of 10-11 rats ±SD
Nonparametric Mann-Whitney test for independent variables
*Statisticcally significant values at **p<0.01 and at *p<0.05*

moderate hypoxia influences the myelin sheet lipids picture and leads to the decrease of the free cholesterol concentration ands diminishes its estrification. Simultaneously, in the works by Adamczewska-Goncerzewicz et al. [1] and Dorszewska and Adamczewska-Goncerzewicz [6] it was shown that placing the animals for 30 min. in the environment consisting of 7% oxygen in the breathing mixture causes not only free cholesterol decrease but also appearance in hypoxic myelin its precursors (desmosterol) and plant sterols (β-sitosterol) likely passing together with food through pathologically altered membranes due to restricted oxygen accessible.

From the literature, it is known [4] that membrane-cholesterol content decrease and appearance in the myelin structure of its sterol precursors might be responsible for altering physicochemical properties and might contribute to disturbances in water and ions membrane transduction through this peptide-lipid structure.

Also it is known that the structure of the hypoxic myelin is changed not only by cholesterol lowered level but also by increased concentration of its esters. According to Wender et al. [23] cholesterol esters are important pattern destroying myelin membrane in different pathological conditions (for example, hypoxia, SM). They are created with acting sterole-ester hydrolase from free membrane cholesterol and from polyunsaturated fatty acids released from the membrane phospholipids [7].

Morover, from the performed experiments it follows that within hypoxic meylin sheet there takes place a decrease of the cerebrosides and sulfatides contents, significant compound of the membrane structures responsible for correct myelin function. These alterations are accompanied by increased level of most membrane phospholipids [23] except plasmalogens. The membrane phospholipids are thought to play an important role in the intracellular signals transduction and to support the fluency and correct membrane structure [4] that is necessary in protection from destruction activity of FOR which are created rapidly in hypoxia conditions. During normal conditions the organism regulates the FOR level with natural enzymatic antioxidants: peroxidase, catalase, superoxide dismutase and antioxidants.

Viatmin E creates the first defence line from peroxidation of polyunsaturated fatty acids presented in the membrane lipids. The performed studies showed that vitamin E administration to the experimental animals before mild experimental hypoxia stabilizes both neutral lipids, by protection of free cholesterol release from the myelin sheet and some phospholipids that are: lysophosphatidylcholine or phosphatidylinositide supporting their balance in the distant periods after the experiment (2 months).

It is thought that the balance of these phospholipids level with the concentration observed in the control animals is connected with returning hypoxic myelin many functions due to proper transport through the membrane and to nervous transduction and also to protection of its normal configuration [13]. We noticed that vitamin E administration to the young experimental animals does not change the membrane lipid picture and that is why it suggests that it might be used as safe pharmacological agent in long-lasting use. In the literature it is indicated that vitamin E given to the patients with arteriosclerotic alterations [10] and with ischemic heart disease [19] protected against changes in vessels of these patients. On the other hand, stabilization of some membrane lipids shown in the performed studies after moderate hypoxia under vitamin E influence and examples from the literature [5, 19] of its positive action in ischemic brain diseases and heart failures may suggest that alpha-tocopherol administration before hypoxia might find clinical indications in practice.

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