Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 15
Sofia • 2010

Lipid Profile of Rat Brain Nuclear and Microsomal Subcellular Fractions during Ischemia

E. Petrova, A. Dishkelov, E. Vasileva

Institute of Experimental Morphology and Anthropology with Museum, Department of Neuromorphology, Bulgarian Academy of Sciences, Sofia

In this study, we report lipid changes in brain nuclei and microsomes in a rat model of cerebral ischemia. We found a decrease of total phospholipids and an increase of total cholesterol and glycolipids in both fractions. Total free fatty acids (FFAs) tended to decrease in microsomes and to increase in nuclei. These changes indicate a disturbance of lipid metabolism and may be interpreted as a physiological adaptive response to ischemia.

Key words: lipids, cerebral ischemia, nuclei, microsomes, rat brain.

Introduction

Among the biochemical events occurring during ischemia, membrane alterations have important consequences on the brain metabolism and function. Despite the intense research on lipid metabolism during cerebral ischemia, little is known about lipid changes in the brain subcellular fractions. The aim of the present investigation is to evaluate the level of phospholipids, cholesterol, glycolipids and free fatty acids in nuclei and microsomes in rat model of cerebral ischemia.

Materials and Methods

Three-month-old male Wistar rats were used in the experiment. Animals were subjected to cerebral ischemia according to the model of S m i t h et al. [8] with minor modifications. Nuclear and microsomal subcellular fractions were isolated according to the method described by V e n k o v [10]. Lipids were extracted according to the technique described by K a t e s [11]. The content of cholesterol and FFAs was determined by gas chromatography as we previously described [5, 6]. The content of total glycolipids was determined according to H a m i l t o n et al. [3]. Total phospholipids were determined by the method of B a r t l e t t [1]. Glycolipid and phospholipid classes were separated by thin-layer chromatography.

The data were analyzed with Student's t-test.

Table 1. Changes of the phospholipid content in nuclei (Nuc) and microsomes (Ms) after cerebral ischemia

Brain fraction		Phosphatidic acid	Lysophospho-lipids	Phosphatidyl-inositol	Sphingo-myelin	Phosphatidyl-serine	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Total phospholipids
Nuc	Control	2.07±0.05	–	0.055±0.01	0.304±0.03	5.249±0.05	13.515±0.06	24.803±0.07	45.995±0.07
	Ischemia	0.786±0.02 $p<0.001$	–	0.668±0.04 $p<0.001$	1.131±0.01 $p<0.001$	6.562±0.04 $p<0.001$	11.048±0.04 $p<0.001$	12.411±0.02 $p<0.001$	32.606±0.08 $p<0.001$
Ms	Control	2.996±0.07	0.261±0.03	–	0.871±0.02	9.763±0.05	20.288±0.07	29.421±0.09	63.599±0.1
	Ischemia	0.735±0.02 $p<0.001$	0.188±0.02 $0.01<p<0.001$	0.563±0.04	2.036±0.04 $p<0.001$	4.459±0.04 $p<0.001$	17.325±0.08 $p<0.001$	12.195±0.08 $p<0.001$	37.501±0.06 $p<0.001$

Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

Results and Discussion

Our results showed a decrease in total phospholipid content by 29% in nuclei and by 41% in microsomes which is due to hydrolysis by different phospholipases (Table 1). The total cholesterol increased 2.4-fold and 7.2-fold in nuclei and microsomes, respectively (Table 2). Ischemia caused nearly 2-fold increase in total FFAs in nuclei and 3.7-fold decrease in total FFAs in microsomes (Table 3). The content of total glycolipids rose 6-fold in nuclei and 20-fold in microsomes (Table 4).

The various changes of the phospholipid classes may be influenced by differences in their turnover. Another possible reason is the difference in the substrate specificity of each phospholipid or in the accessibility of phospholipase A_2 to phospholipids due to a different distribution of each phospholipid in the membrane [2]. In nuclei, 12-fold increase in phosphatidylinositol (PI), and 2.6-fold decrease in phosphatidic acid (PA) were the most pronounced changes. Probably the low content of PA is due to its mobilization in the synthesis of PI or to the action of nuclear PA-phosphohydrolase. In microsomes, all phospholipid classes tended to decrease except for sphingomyelin whose concentration was increased by 134% (Table 1).

The concentration of the free cholesterol was increased 3-fold in nuclei and nearly 2-fold in microsomes. In the latter, we found 22-fold increase in esterified cholesterol (Table 2). The high concentration of sterol esters can apparently be explained with a role of the ester to serve as a carrier and storage site for some toxic free fatty acids [7]. It is reported that the accumulation of cholesterol and cholesterol esters represents a durable adaptive response to different forms of cell injury and there is a striking correlation between the severity of tissue injury and the extent of cholesterol accumulation [9].

The gangliosides increased 6-fold and 12-fold in nuclei and microsomes, respectively, while cerebrosides rose 6-fold and 41-fold in nuclei and microsomes, respectively (Table 4). Probably the high content of cerebrosides makes the membrane steadier because cerebrosides contribute to a dense network of H-bonding between three hydroxy groups of cholesterol, the hydroxy group of the sphingosine, the hydroxy groups of the acyl chains and the amide bond of the sphingolipids. Considering gangliosides as neuroprotectors [4], these changes may be interpreted as a defensive and compensatory mechanism against the ischemic shock.

In nuclei, the concentration of stearic acid ($C_{18:0}$) increased 7-fold (Table 3). In microsomes the content of arachidonic acid ($C_{20:4}$) decreased 2.6-fold and it can be due

Table 2. Changes of the cholesterol content in nuclei and microsomes after cerebral ischemia

Brain fraction		Free cholesterol	Esterified cholesterol	Lano-cholesterol	Total cholesterol
Nuc	Control	0.293±0.06	0.131±0.05	0.005±0.001	0.431±0.05
	Ischemia	0.921±0.06 $p<0.001$	0.102±0.05	–	1.025±0.17 $p<0.001$
Ms	Control	0.392±0.06	0.138±0.02	–	0.531±0.09
	Ischemia	0.708±0.05 $p<0.001$	3.11±0.05 $p<0.001$	–	3.824±0.33 $p<0.001$

Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

Table 3. Changes of the FFAs content in nuclei and microsomes after cerebral ischemia

Brain fraction		$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{20:2}$	$C_{20:4}$	Total
Nuc	Control	0.267±0.02	0.324±0.01	–	0.256±0.02	–	0.435±0.01	0.153±0.02	–	3.471±0.13	4.905±0.18
	Ischemia	–	–	1.73±0.03	1.828±0.04 $p<0.001$	1.501±0.06	–	–	–	3.418±0.13	8.473±0.1 p<0.001
Ms	Control	0.596±0.02	0.056±0.01	–	13.29±0.09	–	0.977±0.04	–	–	6.309±0.05	21.23±0.04
	Ischemia	–	–	0.955±0.02	–	2.325±0.02	–	–	0.112±0.01	2.413±0.11 p<0.001	5.805±0.1 p<0.001

Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

Table 4. Changes of the glycolipid content in nuclei and microsomes after cerebral ischemia

Brain fraction		Gangliosides	Cerebrosides	Total glycolipids
Nuc	Control	0.216±0.02	0.324±0.03	0.54±0.04
	Ischemia	1.363±0.05 p<0.001	2.006±0.05 p<0.001	3.368±0.04 p<0.001
Ms	Control	0.212±0.03	0.082±0.05	0.294±0.04
	Ischemia	2.503±0.04 p<0.001	3.372±0.05 p<0.001	5.875±0.04 p<0.001

Values are expressed in mg/g dry lipid residue/ml.

to its involvement in PI synthesis, oxidation processes, diffusion into cerebral spinal fluid or reesterification. A prominent change was the accumulation of $C_{16:1}$, $C_{18:1}$ and $C_{20:2}$ in both fractions which would contribute to membrane permeability because of the high rate of unsaturation.

In conclusion, our results suggest that the ischemic process causes various lipid changes in nuclear and microsomal brain subcellular structures, directed to enhancement of the functional adaptive possibilities of the brain during ischemia.

References

1. Bartlett, G. R. Phosphorus assay in column chromatography. – J. Biol. Chem., **234**, 1959, No 3, 466-468.
2. Daum, G. Lipids of mitochondria. – Biochim. Biophys. Acta, **822**, 1985, 1-42.
3. Hamilton, P. B. A spectrometric determination of glycolipids. – Anal. Chem., **28**, 1956, 557-565.
4. Mahadik, S. P., S. K. Karpiak. Gangliosides in treatment of neural injury and disease. – Curr. Trends Rev., **15**, 2004, No 4, 337-360.
5. Petrova, E., A. Dishkelov, E. Vasileva. Changes of the cholesterol content in rat brain subcellular fractions in experimental model of cerebral ischaemia. – Compt. Rend. Bulg. Acad. Sci., **58**, 2005, No 7, 839-842.
6. Petrova, E., A. Dishkelov, L. Venkov, E. Vasileva. Changes in free fatty acids in rat brain subcellular fractions after cerebral ischemia. – Acta Morphol. et Anthropol., **11**, 2006, 9-16.
7. Ramsey, R. B., A. N. Davison. Steryl esters and their relationship to normal and diseased human central nervous system. – J. Lipid Res., **15**, 1974, 249-255.
8. Smith, M.-L., G. Bendek, N. Dahlgren, I. Rosén, T. Wieloch, B. K. Siesjö. Models for studying long-term recovery following forebrain ischemia in the rat. 2. A 2-vessel occlusion model. – Acta Neurol. Scand., **69**, 1984, 385-401.
9. Zager, R. A., T. Andoh, W. M. Bennett. Renal cholesterol accumulation. – Am. J. Pathol., **159**, 2001, No 2, 743-752.
10. Венков, Л. Получаване на обогатени фракции на елементи, изграждащи нервната тъкан. – Съвр. пробл. невроморфол., **11**, 1983, 1-60.
11. Кейтс, М. Техника липидологии. Москва, Мир, 1975. 322 с.