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Lipid Composition of Mitochondrial Membrane in Ischemic Rat Brain

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In this study, we report lipid changes in brain mitochondria in a rat model of cerebral ischemia. We found an increase of total phospholipids, cholesterol, glycolipids and free fatty acids (FFAs) with the largest increase of the cholesterol and glycolipids — 14 and 6 times the control values, respectively. These changes indicate a disturbance of lipid metabolism and may be interpreted as a physiological adaptive response to ischemia.

Key words: lipids, cerebral ischemia, mitochondria, rat brain.

Introduction

It has been established that alterations in lipid metabolism are key event that contributes to neuronal death in cerebral ischemia [4]. The studying of pathogenic mechanisms on brain subcellular level is of great interest because membranes and membrane-associated enzymes have a crucial role in energy metabolism. The relationship between the membrane lipid environment and its intrinsic enzymes is well documented in mitochondrial membranes [2]. It is known that mitochondria are responsible for the energy state of the cell. The reduced supply of oxygen during ischemia results in ATP depletion in brain, loss of ion homeostasis, changes in calcium metabolism and release of free radicals [9, 11].

The aim of the present investigation is to evaluate the level of phospholipids, cholesterol, glycolipids and free fatty acids in mitochondrial membrane 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. [12] with minor modifications. Mitochondrial subcellular fraction was isolated according to the method described by Venkov [13]. Lipids were extracted according to the technique

described by K a t e s [14]. The content of cholesterol and FFAs was determined by gas chromatography as we previously described [6, 7]. 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 Perkin-Elmer scanning spectrophotometer was used to estimate the concentration of migrated spots.

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

Results and Discussion

In our study we examined the ischemia-induced changes of four lipid classes in rat brain mitochondria. The total phospholipid content was increased by 6% (from 36.6±0.05 to 38.75±0.16 mg/g/ml, $p<0.001$). The total cholesterol increased 14-fold (from 0.54±0.07 to 7.4±0.17 mg/g/ml, $p<0.001$). Ischemia caused a 41% increase in the content of total FFAs (from 35.67±0.2 to 50.25±0.1 mg/g/ml, $p<0.001$). The content of total glycolipids rose 6-fold (from 0.48±0.05 to 2.88±0.09 mg/g/ml, $p<0.001$). The changes in the percentage of individual phospholipids, sterols, glycolipids and FFAs are given in Fig. 1, 2, 3 and 4, respectively.

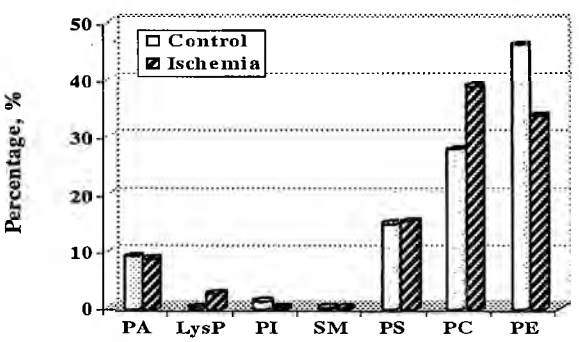

Fig. 1. Changes of the phospholipid content in brain mitochondria after cerebral ischemia. PA — phosphatidic acid, SM — sphingomyelin

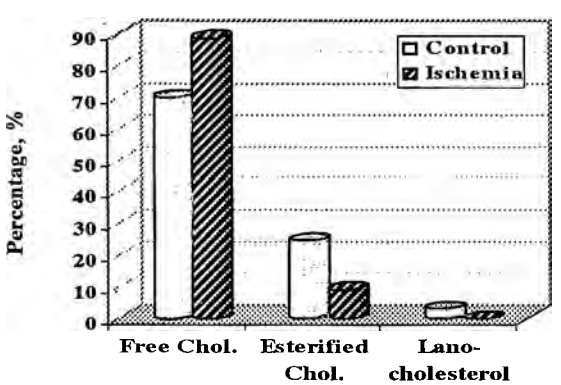

Fig. 2. Changes of the cholesterol content in brain mitochondria after cerebral ischemia

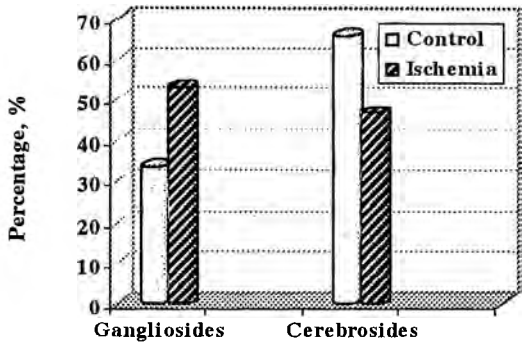

Fig. 3. Changes of the glycolipid content in brain mitochondria after cerebral ischemia

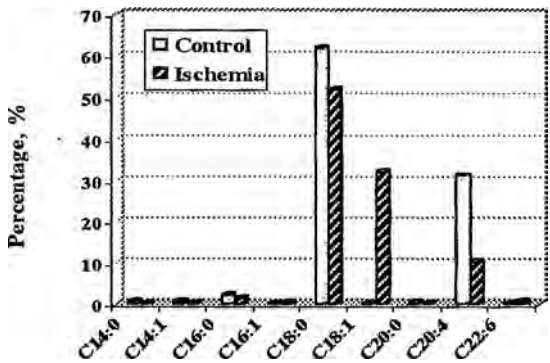

Fig. 4. Changes of the free fatty acid content in brain mitochondria after cerebral ischemia

The content of phosphatidylserine (PS), phosphatidylcholine (PC) and lysophospholipids (LysP) was increased which was most pronounced in LysP — more than 8-fold. The concentration of phosphatidylethanolamine (PE) and phosphatidylinositol (PI) was decreased by 23% and 70%, respectively. Probably the mitochondrial Ca^{2+}-dependent phospholipase A_2 is responsible for the mitochondrial membrane damage, because there are data about its involvement in the turnover of membrane phospholipids and in the process of Ca^{2+} release from mitochondria [8]. PE and PC accounted for 72% of the total phospholipids and it can be suggested that the high amount of unsaturated molecular species in their composition make them appropriate substrates for mitochondrial phospholipase A_2.

The concentration of the free and esterified cholesterol was increased 17-fold and 5-fold, respectively. Probably the hydrolysis of phospholipids disturbs the integrity of the membrane which leads to the release of active cholesterol that can easily be esterified with fatty acid. 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 the otherwise toxic free fatty acids [10].

The content of gangliosides and cerebrosides increased 9-fold and 4-fold, respectively. Gangliosides are considered neuroprotectors [5] and the high content may be interpreted as a defensive and compensatory mechanism against the ischemic shock.

The concentration of $C_{16:0}$ and $C_{18:0}$ was increased by 5% and 18%, respectively. The content of $C_{20:0}$ and $C_{20:4}$ was decreased by 80% and 18%, respectively. A notable observation was the accumulation of $C_{16:1}$ and $C_{18:1}$. We found $C_{22:6}$ too, which appears to be a neuroprotector.

In conclusion, our results reveal that the ischemic process disrupts to a great extent the lipid metabolism in brain mitochondria. These changes are probably associated with impaired energy metabolism and may indicate the disturbances in lipid biosynthesis.

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