Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 12
Sofia • 2007

Changes of the Glycolipid Content in Rat Brain and Brain Subcellular Fractions in An Experimental Model of Cerebral Ischemia

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In this study, we present data from our examinations of the changes of glycolipid content in rat brain and different brain subcellular fractions in a model of cerebral ischemia. In control rats, the total glycolipid content was the highest in nuclei and the lowest in the brain homogenate. Gangliosides and cerebrosides were the major glycolipid classes and they ranged from 34% to 72% and from 28% to 66% of total glycolipids in different subcellular fractions, respectively. Cerebral ischemia led to increase of total glycolipids with the largest increase in the homogenate and microsomes — 89 and 20 times the control values, respectively. The total glycolipid content was the highest in the brain homogenate and the lowest in myelin. The accumulation of glycolipids may be interpreted as a physiological adaptive response to ischemia.

Key words: glycolipids, cerebral ischemia, subcellular fractions, rat brain.

Introduction

Glycolipids are important constituents of cells and their concentration is highest in the nervous system. Beyond structural functions, glycolipids play a variety of biological functions, including cellular recognition and adhesion as well as signalling [1, 9, 12]. These lipids can serve as tumor markers and membrane regulatory factors [3]. A number of studies have shown that gangliosides are immunogenic and they participate in multiple sclerosis pathogenesis [10].

Membrane lipid degradation plays an important role in the pathogenesis of ischemic brain damage, but there is little information on changes in total glycolipids, cerebrosides and gangliosides. The present study was undertaken to evaluate the level of glycolipids in brain homogenate and different subcellular fractions from both normal and ischemic rat brains.

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. [7] with minor modifications.

Brain subcellular fractions were isolated according to the method described by V e n k o v [11]. Lipids were extracted according to the technique described by K a t e s [13]. The content of total glycolipids was determined according to H a m i l t o n et al. [2]. Glycolipid classes were separated by thin-layer chromatography using the following eluate: chloroform:methanol:water 65:25:4 (v/v/v). The Perkin-Elmer scanning spectrophotometer was used to estimate the concentration of migrated spots.

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

Results

The content of total glycolipids in brain homogenate and different subcellular fractions is shown in Table 1. In control rats, total glycolipids had the highest concentration in nuclei (0.54±0.04 mg/g/ml) and the lowest in the brain homogenate (0.181±0.02 mg/g/ml). Gangliosides and cerebrosides were the two main glycolipid classes and they accounted for 34% to 72% and for 28% to 66% of total glycolipids in different subcellular fractions, respectively (Fig. 1).

Table 1. Changes of the glycolipid content in rat brain after cerebral ischemia

Brain fraction		Gangliosides	Cerebrosides	Total glycolipids
Hom	Control	0.068±0.01	0.112±0.01	0.181±0.02
	Ischemia	4.744±0.06 $p<0.001$	11.354±0.06 $p<0.001$	16.098±0.12 $p<0.001$
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 $p<0.001$
Myel	Control	0.107±0.03	0.118±0.03	0.225±0.04
	Ischemia	0.093±0.004 *ns*	0.307±0.02 $p<0.001$	0.4±0.02 $p<0.001$
Syn	Control	0.227±0.04	0.23±0.4	0.457±0.08
	Ischemia	0.443±0.05 $p<0.001$	0.614±0.03 $0.1<p<0.05$	1.057±0.05 $p<0.001$
Mit	Control	0.161±0.05	0.317±0.02	0.478±0.05
	Ischemia	1.526±0.05 $p<0.001$	1.349±0.06 $p<0.001$	2.875±0.09 $p<0.001$

Hom=homogenate; Nuc=nuclei; Ms=microsomes; Myel=myelin; Syn=synaptosomes; Mit=mitochondria. Values are expressed in mg/g dry lipid residue/ml, $n=5$, *ns* – indicates no significant difference.

In the brains of rats subjected to cerebral ischemia, we found increased levels of total glycolipids, gangliosides and cerebrosides in all subcellular fractions (Table 1). The increase of total glycolipids was the highest in the homogenate and microsomes – 89 and 20 times, respectively. Gangliosides made up to 23-53% of the total glycolipids in different fractions (Fig. 1). The brain homogenate contained the highest amounts of gangliosides (4.744±0.06 mg/g/ml) and myelin the lowest (0.093±0.004 mg/g/ml). Cerebrosides accounted for 47 to 77% of total glycolipids in different fractions (Fig. 1). The highest concentration of cerebrosides was observed in the brain homogenate (11.354±0.06 mg/g/ml) and the lowest (0.307±0.02 mg/g/ml) in myelin.

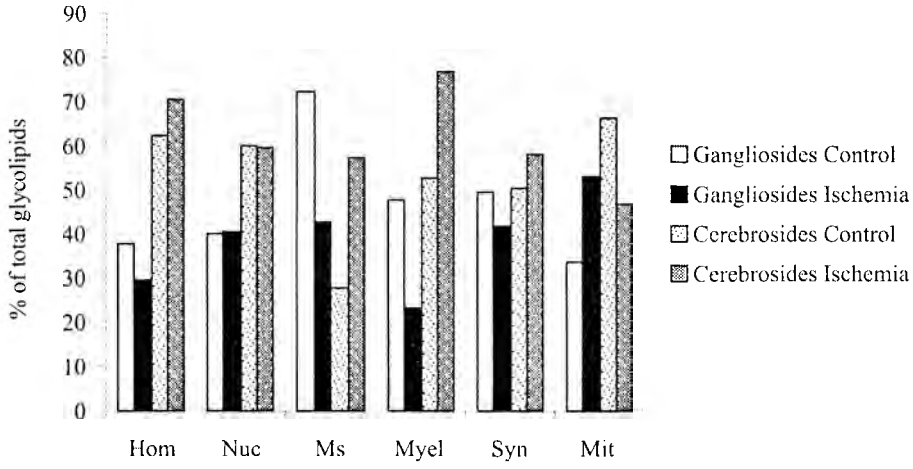

Fig. 1. Changes of gangliosides and cerebrosides in rat brain after cerebral ischemia

Discussion

In this study, we examined the changes in glycolipid content of rat brain homogenate and brain subcellular fractions in an experimental model of cerebral ischemia.

In controls, we found the highest content of total glycolipids in the nuclear fraction and it can be suggested that these lipids influence the membrane-mediated processes in the nuclei. Nuclei contained the highest amounts of cerebrosides in comparison to the homogenate and the other subcellular fractions. Gangliosides in the nuclear membrane probably play a key role in maintaining the nuclear Ca^{2+} homeostasis. Some studies suggest the presence of limited intranuclear pools of gangliosides [4].

Gangliosides are probably synthesized in the membranes of endoplasmic reticulum and this explains the observed high content of gangliosides in microsomal fraction. This proposition is supported by studies on subcellular distribution of the enzymes involved in ganglioside synthesis [9].

In myelin cerebrosides together with the polar head groups of phosphatidylserine and phosphatidylinositol provide a polyanionic surface array. Strong interactions with both the positively charged myelin basic protein at the cytosolic and hydrophobic domains of proteolipid protein at the extracytoplasmic surface might contribute to the tight compaction of the multilayer membrane system [1].

Synaptosomes contained the highest amounts of gangliosides in comparison to the homogenate and the other subcellular fractions and these results are in agreement with previous reports [14]. These data suggest the specific role of gangliosides in synaptic transmission. Gangliosides are thought to be functional in memory formation too [6, 8].

In mitochondria gangliosides probably influence the Ca^{2+} homeostasis and ionic balance and contribute to the high permeability of the mitochondrial membrane.

In the brains of rats subjected to cerebral ischemia, we found an increase in both gangliosides and cerebrosides in all subcellular fractions. As a result the total glycolipids estimated increased from 2 to 89 times in different fractions. The high concentration of glycolipids and especially gangliosides can apparently be explained by their neuroprotective effect. It is supposed that gangliosides can acutely reduce the extent of CNS injury by protection of membrane structure and function [5]. Another hypothesis sup-

ports the view that gangliosides may promote neuronal regeneration through modulation of trophic factors.

The high content of cerebrosides after ischemia probably makes the membranes steadier and it appears to be a protective and compensatory mechanism against ischemic damage. Most probably, 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 [1].

In conclusion, the present results show that cerebral ischemia disrupts to a great extent the brain lipid metabolism and in particular the glycolipid metabolism. The accumulation of glycolipids may indicate the energy disturbances and concomitant restriction of glucose supply to the ischemic brain and may be interpreted as a physiological adaptive response to ischemia.

Acknowledgements: This study was supported by National Science Fund of Bulgaria (NSFB) under Contract MU-L-1512/05.

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