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Effect of Hypoxia on Glycolipids in Rat Brain Myelin

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In this study we present data from our examinations of the changes in myelin glycolipid content in a rat model of sodium nitrite-induced hypoxia. Twenty male Wistar rats at the age of three months were used in the experiment. The myelin fraction was isolated and lipids were extracted. The glycolipid content was measured by spectrophotometry and thin-layer chromatography.

In the myelin of hypoxic brains, we found increased levels of total glycolipids (2.4-fold), gangliosides (2.1-fold), and cerebrosides (2.7-fold). These changes indicate a disturbance of lipid metabolism. The accumulation of glycolipids may be interpreted as a physiological adaptive response to hypoxia.

Key words: gangliosides, cerebrosides, sodium nitrite, hypoxia, myelin, rat brain.

Introduction

Brain is of special interest for hypoxia studies as it is critically dependent on its oxygen supply. Hypoxia, as well as ischemia, provokes alterations in the lipid metabolism. Although considerable efforts have been directed at evaluating alterations in hypoxia, lipid metabolism at brain subcellular level has not been fully evaluated.

It is well recognized that the myelin sheath of the brain is a structure which is highly sensitive to hypoxic injury. Glycolipids are a dominant class of lipids in the myelin bilayer. Therefore, the present investigation was undertaken to evaluate the level of glycolipids in rat brain myelin in a model of sodium nitrite-induced hypoxia.

Materials and Methods

Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to sodium nitrite-induced hypoxia. Sodium nitrite was administered intravenously at 20 mg/kg body weight (2 ml/kg dosing volume). Hypoxic rats were killed by decapitation.

Myelin was isolated according to the method described by Venkov [12] using two-step sucrose gradient. Lipids were extracted according to the method of Kates [13] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v). The content of total glycolipids was determined according to Hamilton et al. [5]. Glycolipid classes were separated by thin-layer chromatography.

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

The animal experiments were performed in accordance with animal protection guidelines approved by the Ethics Committee for experimental animal use at IEMPAM – BAS.

Results and Discussion

In the present study, we examined the changes in myelin glycolipids in a rat model of sodium nitrite-induced hypoxia.

It is well known that the lipid bilayer of myelin membranes has highly specialized properties as a result of its unique lipid composition [10]. A dominant class of lipids in the myelin bilayer are the glycolipids, which include cerebrosides, sulfatides and gangliosides. Cerebrosides are the predominant component and they 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 [2]. Our observations in control rats are in good agreement with the literature data. We found gangliosides and cerebrosides and they accounted for 47.5% (0.107±0.03 mg/g dry lipid residue/ml; mg/g/ml) and for 52.5% (0.118±0.03 mg/g/ml) of total glycolipids, respectively.

Hypoxia is one of the major pathological conditions causing neuronal cell injury. The brain is the most hypoxic vulnerable of all vertebrate tissues because of its high rate of aerobic metabolism. In our experiments we applied a model of sodium nitrite-induced chemical hypoxia. This model is convenient because no restraint of the animal or special enclosure is required [3]. It refers to anemic hypoxia – a condition in which there is a reduction in hemoglobin's ability to transport oxygen. Sodium nitrite converts hemoglobin to methemoglobin and unlike ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of the blood is reduced. It is reported that the oxidation of oxyhemoglobin by nitrite to produce methemoglobin is a complex process that has been characterized by a lag phase followed by an autocatalytic phase [7].

In hypoxic brains we found increased levels of total glycolipids (2.4-fold), gangliosides (2.1-fold), and cerebrosides (2.7-fold) (Fig. 1). Gangliosides and cerebrosides accounted for 41% (0.224±0.01 mg/g/ml) and for 59% (0.317±0.03 mg/g/ml) of the total glycolipids, respectively. 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 central nervous system injury by protection of membrane structure and function [8]. Another hypothesis supports the view that gangliosides may promote neuronal regeneration through modulation of trophic factors.

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 [2]. Considering gangliosides as neu-

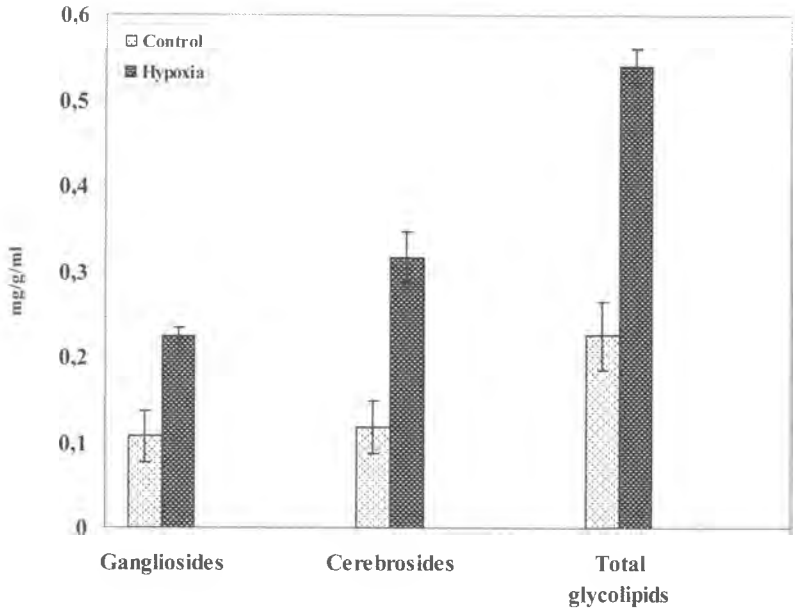

Fig. 1. Changes of the gangliosides, cerebrosides and total glycolipids in hypoxic rat brain myelin. Values are expressed in mg/g dry lipid residue/ml, $p < 0.001$

roprotectors [8], these changes may be interpreted as a defensive and compensatory mechanism against hypoxic damage.

There are few reports in the literature on glycolipid changes in hypoxic rat brain. To our knowledge, only the study of Baev et al. [1] refers to sodium nitrite-induced hypoxia although performed on total brain homogenate. Our data are in good agreement with the latter as it demonstrates elevated content of rat brain gangliosides. In contrast, earlier studies of Domanska-Janik et al. [4] show moderate decreases in cerebrosides and gangliosides of brain homogenate in three different experimental models of oxygen deficiency. A decrease in the myelin cerebrosides in moderate hypoxia is documented by Kapelusiak-Pielok et al. [6], too. Similar results are demonstrated by Ramirez et al. [9] in neonatal hypoxia-ischemia in the rat hippocampus. Their findings indicate reduced ganglioside content. Besides, no effect of hypoxia on the content of myelin cerebrosides is observed by Wender et al. [11]. As we demonstrate the opposite pattern of changes in the glycolipid content, our results differ from the above literature data. Most probably it is due to the different types of hypoxia, degree and duration of hypoxia, the time interval after which the studies are performed, the regional and subcellular fractions studied, etc.

Conclusion

Our data provide evidence that sodium nitrite-induced hypoxia influences glycolipid metabolism in rat brain myelin. They also show that myelin responds to hypoxia by synthesizing a high amount of cerebrosides and gangliosides and this is probably involved in the cell survival pathways.

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