GLYCOLIPID CONTENT OF THE NUCLEAR MEMBRANE IN HYPOXIC RAT BRAIN

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ABSTRACT

In this study, we report glycolipid changes in the nuclear subcellular fraction from hypoxic rat brains. Male Wistar rats at the age of three months were subjected to sodium nitrite -induced hypoxia. The nuclear fraction was isolated and lipids were extracted. The glycolipid content was measured by spectrophotometry and thin-layer chromatography.

In controls, gangliosides and cerebrosides were the major glycolipid classes. In the nuclear membrane of hypoxic brains, we found increased levels of total glycolipids (5.2-fold), gangliosides (4.1-fold), and cerebrosides (5.9-fold). These changes reflect the disturbances in the glycolipid turnover. Most probably they are associated with impaired energy metabolism. The accumulation of glycolipids may be interpreted as a physiological adaptive response to hypoxia.

Keywords: glycolipids, hypoxia, sodium nitrite, nuclear subcellular fraction, rat brain

INTRODUCTION

Hypoxia is an important factor that provokes numerous physiological and pathological responses. The brain is highly sensitive to reduced oxygen levels and it requires nearly constant supply to prevent damage. It is known that brain hypoxia generates a series of biochemical events with several cellular and functional consequences, such as plasma membrane structural damage and delayed cell death (11).

The lipid fraction is particularly sensitive to hypoxia, as compared to other macromolecular compounds. The glycosphingolipids (glycolipids) are a large and heterogeneous family of amphiphatic lipids anchored to the extracytosolic leaflet of cell membranes through the ceramide moiety. At the plasma membrane, they expose their sugar chains out from the cell surface, contributing to the complexity of the glycocalix. Gangliosides and cerebrosides are the main large groups of glycolipids, and they are present in high concentration in the neuronal membranes. Glycolipids play crucial modulatory roles in various cellular functions, including cellular growth and differentiation, cell-cell recognition, adhesion, signal transduction, adaptation of plasma membrane to environmental variations and cell death (19).

The aim of the present investigation was to establish the changes in the level of total and individual glycolipids in the nuclear subcellular fraction from hypoxic rat brains.

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 as we have previously reported (13). Sodium nitrite was administered intravenously at 20 mg/kg body weight. Hypoxic rats were anaesthetized and decapitated 24 h after the administration.

The nuclear fraction was isolated according to the method described by Venkov (16). Brains (except cerebellum) were homogenized in ice-cold 0.32 M sucrose in a glass homogenizer with Teflon pestle and 10% homogenate was made. The homogenate was centrifuged at 3000 g (1500 rev/min) for 10 min. The nuclear fraction was collected. Lipids were extracted according to Kates (8) using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v). Perkin-Elmer scanning spectrophotometer was used to estimate the concentration of migrated spots.

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

The data were analyzed with Student’s t-test. Results are reported as mean values±SEM.

RESULTS AND DISCUSSION

The pathogenesis of cerebral hypoxia has been associated with a time-dependent cascade of molecular events including rapid fall in intracellular
The brain is particularly susceptible to hypoxic oxidative injury because of its abundant lipid content and its limited antioxidant defense systems (5). Several experimental models have been used to recapitulate the human cerebral hypoxia syndrome. In our experiments we applied a model of sodium nitrite-induced hypoxia. Sodium nitrite is reported to stimulate oxidation of ferrous ions in oxyhemoglobin to form methemoglobin as well as various ROS (4). Unlike the ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of the blood is reduced. The administration of sodium nitrite in high concentrations may cause brain inflammation, ischemia and impaired cerebral energy (20).

In this study, we examined the changes in the glycolipid content of the rat brain nuclear subcellular fraction following sodium nitrite-induced hypoxia. We found significant increase in the level of total glycolipids from 0.54±0.04 to 2.796±0.09 mg/g/ml (mg glycolipids per g dry lipid residue per ml lipid extract), p<0.001. The content of gangliosides and cerebrosides was 4.1 times the control value (from 0.216±0.02 to 0.892±0.08 mg/g/ml) and 5.9 times the control value (from 0.324±0.03 to 1.903±0.04 mg/g/ml), respectively (Fig. 1). The glycolipid composition of the rat brain nuclear subcellular fraction following hypoxia is poorly investigated. Most of the studies concern the total brain homogenate. The effect of hypoxic hypoxia on glycolipids is studied in the brain cortex and a reduction in the glycolipid content is reported (2). It is suggested that hypoxia could have activated neuraminidase and change directly the content of ganglioside-bound sialic acid on the synaptic plasma membrane. Neonatal hypoxia/ischemia is reported to reduce ganglioside content in the rat hippocampus (14). In contrast to the above studies, we explored the effect of sodium nitrite-induced hypoxia on glycolipids at brain subcellular level and our findings demonstrate an increase in the glycolipid content in the nuclear fraction.

Most probably the high content of gangliosides is associated with their neuroprotective effect although some gangliosides are highly expressed in pathological conditions (10). It is supposed that gangliosides may promote neuronal regeneration through modulation of trophic factors (14, 18). The high content of cerebrosides after hypoxia makes the membranes steadier and it appears to be a protective and compensatory mechanism against hypoxic 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).

![Fig. 1. Changes of the glycolipid content in rat brain nuclear fraction after sodium nitrite-induced hypoxia. p<0.001](image)

It is known that oxidative stress and reactive oxygen species increase the transcription of a variety of genes participating in compensatory mechanisms that support cell survival in a potentially lethal microenvironment. The hypoxia-inducible factor 1 (HIF-1) and the nuclear factor kappa-B (NFkB) are believed to be differentially regulated by oxidative conditions. They have been identified as a critical component of the cellular and systemic response to hypoxic stress (15, 17). These factors mediate oxygen-dependent transcription of target genes involved in the regulation of adaptive responses, such as changes in energy metabolism and angiogenesis. The nuclear factor kappa-B exists in the cytoplasm in an inactive form. When activated, this factor interacts with the nuclear import machinery and it is translocated to the nucleus, where it binds to the promoter of target genes to initiate transcription (9). There is evidence that certain lipids, such as 18:2n-6, can activate NFkB (7). We have previously reported that the free fatty acid pool of the nuclear fraction is increased following sodium nitrite-induced hypoxia (12). Therefore it is of great importance to study the effect of hypoxia on the brain lipids, especially at brain subcellular level. It would elucidate the cellular and the molecular mechanisms that are involved in the hypoxic injury.

**Conclusions**

Sodium nitrite-induced hypoxia results in glycolipid accumulation in the brain nuclear subcellular fraction. The high levels of gangliosides and cerebrosides indicate the energy disturbances and may represent an adaptive response to this form of brain injury.
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REFERENCES