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EFFECT OF CHEMIC HYPOXIA ON THE PHOSPHOLIPID CONTENT OF RAT BRAIN SYNAPTOSOMES*

UTICAJ HEMIJSKE HIPOKSIJE NA SADRŽAJ FOSFOLIPIDA U SINAPTOZOMIMA U MOZGU PACOVA*

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Ključne reči
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Abstract

In this study, we report changes in the phospholipid content of synaptosomes from hypoxic rat brains. Twenty male Wistar rats at the age of three months were subjected to sodium nitrite-induced hypoxia. The synaptosomal fraction was isolated and the phospholipids were measured by spectrophotometry and thin-layer chromatography.
In controls, phosphatidylcholine and phosphatidylethanolamine were the most prominent components and together they accounted for 71.6% of the total phospholipids in synaptosomes. In the hypoxic brains, the total phospholipids increased by 83%. The different phospholipid classes were not equally affected. Hypoxic synaptosomes showed higher content of lysophospholipids, phosphatidylinositol, phosphatidylserine and phosphatidylcholine and lower concentration of phosphatidic acid. Nevertheless, phosphatidylcholine and phosphatidylethanolamine were the major components after hypoxia.

INTRODUCTION

Phospholipids are an integral component of the mammalian cellular membranes and the most abundant membrane lipids. In the central nervous system, the phospholipids constitute about 70% of the total lipids in the gray matter. Some neurological disorders and pathological conditions are associated with impaired lipid metabolism, including phospholipid metabolism [1, 2]. Phospholipids are particularly sensitive to hypoxia due to the polyunsaturated fatty acids in their composition. In the synaptosomal membrane, they account for the high degree of unsaturation which makes the synaptosomes very susceptible to insufficient oxygen supply and the subsequent oxidative stress [3, 4]. As the membrane lipid environment is essential for the development and regulation of the synaptic functions, it is of great interest to study the effect of hypoxia on the phospholipid content of rat brain synaptosomes.

MATERIAL AND METHODS

Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to sodium nitrite-induced hemic hypoxia as we have previously reported [5]. Sodium nitrite was administered intravenously at 20 mg/kg body weight. Hypoxic rats were lightly anesthetized and sacrificed by decapitation.

Synaptosomal fraction was isolated according to the method described by Venkov [6] using discontinuous two-step sucrose gradient. Lipids were extracted according to the method of Kates [7] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v).

Total phospholipids were measured spectrophotometrically at 820 nm [8]. All major phospholipid classes were separated by thin-layer chromatography using eluate from chloroform:methanol:water 65:25:4 (v/v/v). Perkin-Elmer scanning spectrophotometer was used to estimate the concentration of migrated spots.

Results are reported as mean values ± SD and statistically analyzed by Student's t-test.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

RESULTS AND DISCUSSION

Mammalian cell membranes contain more than thousand different phospholipids [9]. In the brain, four major classes of phospholipids are found [2]. Besides their structural role in the neural membranes, phospholipids have a variety of biological functions. They serve as precursors for various second messengers, function as an energy reservoir and may be involved in many important processes such as apoptosis and cell signaling [1, 2, 9]. Phospholipids are required for the proper function of integral membrane proteins, receptors, and ion-channels [10]. Furthermore, they are amphipathic molecules, asymmetrically distributed in the bilayers. Studies have indicated that this asymmetry may play critical roles in many important biological and cellular processes [2].

Our findings in controls showed the presence of phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylserine (PS) and lysophospholipids (LysP) in the synaptosomal phospholipid composition (Fig. 1). In fact, different types of mammalian cells and tissues, as well as the cellular organelles, have characteristic phospholipid composition which is closely related to their functional activity [9]. Synaptosomal membrane is the major constituent of presynaptic boutons and takes part in mechanisms of the neuronal plasticity and signal transmission [11]. The dynamic processes in the synapses require phospholipids with high metabolic activity. Phosphatidylcholine and phosphatidylethanolamine were the predominant components in the synaptosomes and they accounted for 41.6% and 30% of the total phospholipids, respectively. Some authors report almost equal quantity of PE and PC in the synaptosomal membrane of rats [12]. Moreover, studies on the fatty acid composition of these phospholipids demonstrate that synaptosomal PE contains much more unsaturated fatty acids than PC [12, 13]. The PS content in synaptosomes was also significant and this is in agreement with available literature data [14]. The presence of phosphatidylinositol, though in small concentration, is associated with its implication in the regulation of membrane permeability and synaptic transmission in neurons.

The phospholipid components of the synaptosomal membrane are rich in unsaturated fatty acids whose double bonds are especially susceptible to free radical-initiated oxidation [15]. Oxidative stress, due to disturbance in oxidant-/antioxidant balance, has been implicated as a key mechanism that contributes to tissue damage in hypoxia. The reduced oxygen supply is a pathological condition that may cause neuronal cell injury, neurodegeneration and cell death. Our observations in a rat model of sodium nitrite-induced hypoxia clearly demonstrate changes in the synaptosomal phospholipid composition. Sodium nitrite is commonly used for induction of hemic hypoxia in experimental animal models. It 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.

In the brains of experimental rats subjected to hypoxia, the total phospholipids increased by 83% (from 35.8±0.17 mg/g/ml (mg phospholipids per g dry lipid residue per ml lipid extract) to 65.61±0.12 mg/g/ml). These results differ from the literature data about reduced phospholipid content due to membrane lipid degradation [16]. It is known that synaptosomes are characterized by active transport of ions across their membranes and a high rate of metabolism. Probably such metabolically active structures recover quickly after hypoxic shock. The process of phospholipid synthesis is more intensive than the destructive one and it is directed to restore the integrity of the membrane. The content of the individual phospholipids was also increased except for PA whose concentration decreased 3.8-fold (from 3.63±0.07 to 0.95±0.06 mg/g/ml). The concentration of LysP, PI, PS, PC and PE increased 4.8-fold (from 0.62±0.03 to 3.01±0.05 mg/g/ml), 8-fold (from 0.69±0.04 to 5.51±0.06 mg/g/ml), 2.4-fold (from 5.23±0.08 to 12.4±0.04 mg/g/ml), 1.7-fold (from 14.89±0.1 to 25.41±0.08 mg/g/ml) and 1.7-fold (from 10.74±0.07 to 17.98±0.09 mg/g/ml), respectively (Fig. 1). The inhomogeneous changes of the various classes may be influenced by differences in their turnover. The lower levels of PA following hypoxia were expected as this important intermediate is the main precursor of all neural membrane phospholipids. The higher content of PC may be due to sequential methylation of PE, catalyzed by phosphatidylethanolamine N-methyltransferase. This is one of the pathways for the synthesis of PC and it is reported to be most intensive in synaptosomes [17]. A notable observation was the increase of lysophospholipids. Lysophospholipids may be generated by the action of phospholipases and either hydrolyzed by lysophospholipase or used to regenerate phospholipids in the remodeling pathway. They have recently shown to be involved in many physiological and pathological processes such as inflammation, reproduction, angiogenesis, tumorogenesis, atherosclerosis and nervous system regulation [18]. It is reported that high concentrations of these metabolites may not only alter the physicochemical properties of neuronal cell membranes, but also can lead to the loss of ionic gradients due to alterations in the conformation and function of transmembrane ion-channels, inflammation, and oxidative stress [2]. Small amounts of sphingomyelin (SM) were also detected in the synaptosomal phospholipid composition following hypoxia (0.35±0.03 mg/g/ml).

The changes in the phospholipid composition of synaptosomes after hypoxia may result in altered phospholipid asymmetry, therefore disturbances in the membrane stability, fluidity and permeability [19]. There are clues to a decrease in the number of synaptic connections within the cortex of hypoxia exposed rats [20]. Studies demonstrate ultrastructural changes in the synaptosomal membrane as lesions of the outer membrane and loss of the synaptoplasmatic content, or condensation of the synaptosomal content with increasing electron density, deformation of the synaptosomes in size and shape, disappearance of structural details, degeneration of intrasynaptosomal mitochondria in form of strong condensation and, finally, formation of intrasynaptosomal vacuoles with dense core inclusions [20].

In conclusion, our data reveal that sodium nitrite-induced hypoxia provokes changes in the phospholipid composition of brain synaptosomes. These alterations are probably associated with impaired energy metabolism. Nevertheless, they may be indicative of a high rate of recovery processes in the metabolically active synaptosomal membrane.

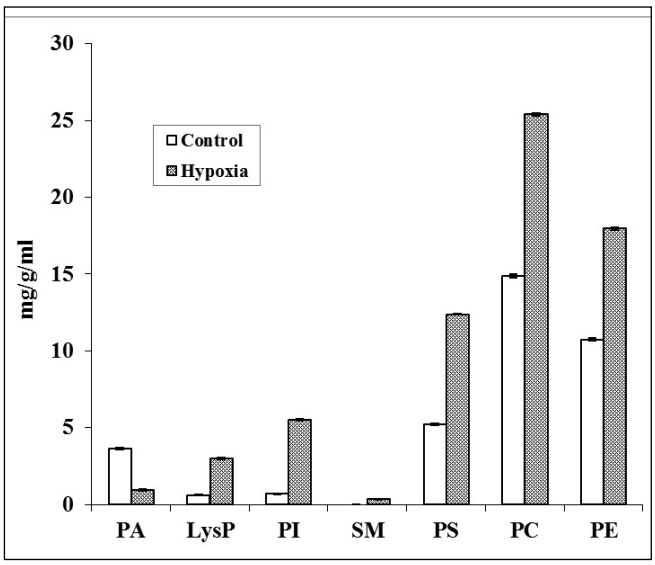

Fig. 1. Changes of the phospholipid classes in rat brain synaptosomes following hemic hypoxia. Data are reported as mean values ± SD and expressed in mg/g dry lipid residue/ml. p< 0.001

Sažetak

U radu prikazujemo promene u sastavu fosfolipida nastale u mozgu pacova u hipoksiji. Dvadeset mužjaka Wistar pacova od tri meseca podvrgnuti su hipoksiji indukovanoj natrijum nitritom. Izolovana je sinaptozomalna frakcija i fosfolipidi su mereni primenom spektrofotometrije i tankoslojne hromatografije.

U kontrolama, fosfatidil holin i fosfatidil etanol amin bile su najistaknutije komponente i ukupno ih je bilo 71,6% od ukupnih fosfolipida u sinaptozomima. U hipoksičnom mozgu ukupni fosfolipidi porasli su za 83%. Različite klase fosfolipida nisu bile podjednako zahvaćene. U hipoksičnim sinaptozomima bio je povečan sadržaj lizofosfolipida, fosfatidil inozitola, fosfatidil serina i fosfatidil holina, a smanjena koncentracija fosfatidilne kiseline. Ipak, fosfatidil holin i fosfatidil etanol amin su bile glavne komponente nakon hipoksije.

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