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Effect of hemic hypoxia on the cholesterol content of rat brain synaptosomal membrane

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In this study we report changes in the cholesterol content of rat brain synaptosomes following hemic hypoxia. Male Wistar rats at the age of three months were subjected to sodium nitrite-induced hemic hypoxia. The synaptosomal fraction was isolated and lipids were extracted. The cholesterol content was measured by gas-liquid chromatography.

In the synaptosomes of hypoxic brains, we found increased levels of total cholesterol (10.8-fold), free cholesterol (6-fold), and esterified cholesterol (26-fold). These changes indicate a disturbance of cholesterol homeostasis. The cholesterol accumulation may be interpreted as a physiological adaptive response to hypoxia.

Key words: cholesterol, synaptosomes, rat brain, hemic hypoxia

Introduction

Cholesterol is the main mammalian membrane-active sterol, and also the most studied one. It is largely present in the plasma membranes of glial cells and neurons and in the specialized membranes of myelin. Among the variety of biological functions, cholesterol is suggested to enhance the production of presynaptic components including synaptic vesicles [12] and release sites [4].

The insufficient oxygen supply has been shown to promote cholesterol accumulation in cells [5] but little is known about the cholesterol subcellular distribution in the hypoxic brain. As the membrane lipid environment is essential for the development and regulation of the synaptic functions, it is of great interest to study how the synaptosomal cholesterol pool is affected by hypoxia.

Materials and Methods

Three-month-old male Wistar rats were subjected to sodium nitrite-induced hypoxia. Sodium nitrite was administered intravenously at 20 mg/kg body weight. Hypoxic rats were lightly anesthetized and sacrificed by decapitation.

The synaptosomal fraction was isolated by discontinuous two-step sucrose gradient centrifugation of rat brain homogenate according to the method of Venkov [15]. Lipids were extracted according to the method of Kates [16] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v).

The cholesterol content was determined by gas-liquid chromatography as we have previously described [7].

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

In the present study, we examined the effect of hemic hypoxia on the cholesterol content of rat brain synaptosomal membrane.

The appearance of sterols in biological membranes is considered to be an important step in membrane evolution [1]. Cholesterol is the main sterol present in the animal tissues. It is required for many physiological functions such as cell membrane construction, cell proliferation and biosynthesis of vital hormones [5]. The level of cholesterol in the membrane strongly affects the barrier properties of membranes as well as many other physicochemical properties of lipid bilayers. Cholesterol regulates the activity of membrane-bound transporters, ion channels, signalling molecules, and transport vesicles [3].

Cholesterol levels strongly influence the establishment and maintenance of synaptic connections and the basic synaptic processes and plasticity [8, 9]. A number of investigators report that cholesterol turnover is most intensive in synaptosomes and support the hypothesis about local synthesis of cholesterol in the synaptic terminals. The majority of brain cholesterol is in unesterified form [13] which finds good support in our observations in control rats. Our findings in control rat brains indicated the predominant presence of free cholesterol (0.67±0.13 mg/g dry lipid residue/ml; mg/g/ml) in the synaptosomal fraction. Esterified cholesterol (0.23±0.02 mg/g/ml), as well as small amounts of lanosterol (0.03±0.001 mg/g/ml) were also found.

Cholesterol homeostasis is very important for human and animals. The dynamic equilibrium between free and esterified cholesterol in the brain is controlled by the activity of the respective sterol ester hydrolases. It is reported that hypoxia may disturb this balance and promote cholesterol accumulation in cells. Moreover, selective sensitivity of synaptosomal membrane function to cerebral cortical hypoxia is demonstrated [10].

In our experiments we applied a model of sodium nitrite-induced chemical hypoxia. It refers to hemic hypoxia – a condition in which there is a reduction in hemoglobin's ability to transport oxygen. Sodium nitrite converts hemoglobin to methemoglobin and unlike the ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of the blood is reduced. As a result, the total cholesterol significantly increased in the synaptosomal subcellular fraction (from 0.93±0.1 to 9.988±0.12 mg/g/ml). The levels of free and esterified cholesterol were 6-fold (4.04±0.06 mg/g/ml) and 26-fold (5.95±0.08 mg/g/ml) above the control values, respectively (Fig. 1). The esterified cholesterol pool accounted for 60% of the total cholesterol. We have previously reported similar changes of the mitochondrial cholesterol content in the hypoxic and ischemic rat brain [6, 7]. Obviously, the hydrolysis of membrane phospholipids during hypoxia disrupts the integrity of the

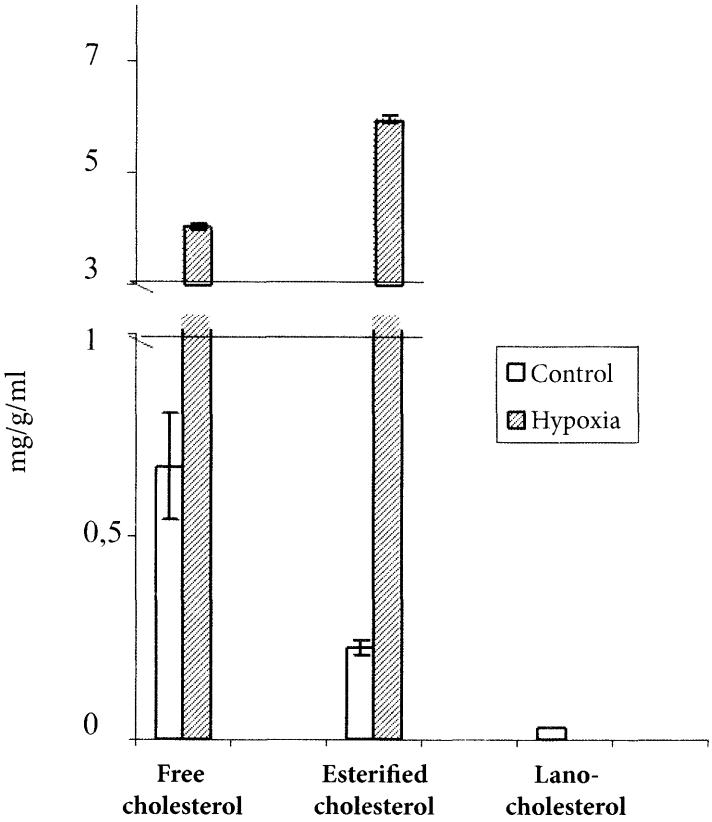

Fig. 1. Synaptosomal cholesterol content of hypoxic rat brain. Values are expressed in mg/g dry lipid residue/ml. $p<0.001$

membrane which can lead to the release of active cholesterol whose esterification can be stimulated by free fatty acids.

The cholesterol accumulation in cells is considered to depend on the balance of complex factors: the extracellular cholesterol uptake via the LDL (low-density lipoprotein) receptor pathway, de novo cholesterol synthesis, intracellular cholesterol trafficking (among organellar, cytosolic, and plasma membrane pools), cellular efflux, and cholesterol esterification/deacylation reactions [2]. Under hypoxic conditions, the activity of acyl-CoA:cholesterol acyltransferase (ACAT) is increased, which leads to a decrease in cholesterol efflux and cholesterol is accumulated in the esterified form [5]. Lipoprotein uptake by LDL receptors is not shown to play an important role in cellular cholesterol accumulation.

The high concentration of sterol esters can apparently be explained with the role of the ester to serve as a carrier and storage site for the otherwise toxic free fatty acids. It is reported that the accumulation of cholesterol and cholesterol esters represents a durable adaptive response to different forms of cell injury [14]. Nevertheless, excess cholesterol must be avoided because it can form solid crystals which can be toxic to cells. Excess cholesterol can also cause many adverse effects such as atherosclerosis or neurological degenerative disease [11].

In conclusion, our data provide evidence that sodium nitrite-induced hypoxia affects the cholesterol content in rat brain synaptosomal membrane which results in

its accumulation. These changes indicate the disturbances in cholesterol biosynthesis, intercellular transport and intracellular distribution of cholesterol, and may be implicated in the cell survival pathways.

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