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LIPID COMPOSITION OF SYNAPTOSOMAL AND MYELIN MEMBRANES IN ISCHEMIC RAT BRAIN

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Abstract

In this study, we report lipid changes in myelin and synaptosomes in a rat model of cerebral ischemia. Three-month-old male Wistar rats were subjected to cerebral ischemia according to the model of Smith with minor modifications. Synaptosomal and myelin fractions were isolated and lipids were extracted. The lipid content was measured by thin-layer chromatography, gas-liquid chromatography and spectrophotometrically.

In the brains of rats subjected to cerebral ischemia, we found an increase of total cholesterol and total glycolipids in both fractions. The content of total free fatty acids (FFAs) and total phospholipids increased in synaptosomes, whereas these lipids tended to decrease in myelin. The changes indicate a disturbance of lipid metabolism and may be interpreted as a physiological adaptive response to ischemia.

INTRODUCTION

A number of studies on animal brains with experimentally induced ischemia have been reported. Most of these studies have focused on the impaired cerebral metabolism. It is known that a short period of interrupted cerebral blood flow restricts the delivery of oxygen and glucose and impairs the energetics required to maintain the ion gradients. Energy failure, free radical, cytokine, and excitatory aminoacid release are considered to contribute to neuronal death [1].

Among the biochemical changes during ischemia, membrane lipid degradation plays an important role in the pathogenesis of ischemic brain damage. Released glutamate stimulates neuronal receptors, resulting in elevated intracellular Ca2+ and activation of phospholipases C and A2. The activation of both two key enzymes in the catabolism of membrane phospholipids, and of diglyceride lipases and other lipases, lead to hydrolysis of the phospholipids and subsequent release of fatty acids. Free fatty acids are known to impair mitochondrial function and to inhibit the Na+, K+-ATP activity [2]. These data suggest a link between lipid changes and ATP depletion.

In this connection, the study of pathogenic mechanisms on subcellular level is of great interest because membranes and membrane-associated enzymes have a crucial role in energy metabolism. However, there are not enough data about lipid changes in myelin and synaptosomes during cerebral ischemia.

The present study was undertaken to evaluate the level of phospholipids, cholesterol, glycolipids and free fatty acids in synaptosomal and myelin membranes in an experimental rat model of cerebral ischemia.

MATERIAL AND METHODS

Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to cerebral ischemia according to the model of Smith [3] with minor modifications. This model is appropriate for studying phospholipid and energy metabolism in ischemia and, more recently, to evaluate neurotransmitter metabolism.

Synaptosomal and myelin fractions were isolated according to the method described by Venkov [4] using two-step sucrose gradient. Lipids were extracted according to the method of Kates [5] using the following eluates: chloroform: methanol 1:2 (v/v) and chloroform:methanol: water 1:2:0.8 (v/v/v).

Total phospholipids and glycolipids were measured spectrophotometrically at 820 nm [6] and at 490 nm [7], respectively. All major phospholipid and glycolipid 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.

The FFAs and cholesterol content was determined by gas-liquid chromatography. A gas chromatograph with

flame ionization detector and connected with Trio Vector computing integrator was used. The fatty acids were converted to fatty acyl methylesters by addition of 25% hydrochloric acid. Hexamethyldisilazane was used as methylation reagent for cholesterol determination. The analysis was performed by injecting 5 µl of the sample into SE-35 column. The temperature was programmed from 85ºC to 205ºC (2.5ºC/min) for FFAs analysis and from 145ºC to 215ºC (2.5ºC/min) for cholesterol analysis. Nitrogen was used as carrier gas at a flow-rate of 40 ml/min.

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

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

RESULTS AND DISCUSSION

The brain contains a large amount of lipids, especially phospholipids which are a major structural component of biological membranes. The analysis of myelin and synaptosomes in control rats demonstrated that both fractions are composed of the following phospholipids: phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM) and phosphatidylserine (PS). Phosphatidylcholine and PE were the most prominent components in myelin and synaptosomes and they accounted for 72% of total phospholipids in both fractions (Table 1). Besides, we found lysophospholipids (LysP) in synaptosomes. Probably LysP are involved in the modulation of pre- and postsynaptic ion channels activity, and they may play a role in the neurotransmission.

Our results showed a significant amount of PA and PI in synaptosomes but the presence of these lipids is not surprising. It is known that synaptic vesicles are a primary site for neurotransmitter storage within the nerve endings. Earlier studies have demonstrated active metabolism of PI and PA in synaptic vesicles and it is suggested the involvement of phosphoinositides in the vascular neurotransmitter release process [8, 9].

Cholesterol is an essential membrane component and the interaction of its hydrophobic rings with the fatty acyl chains of membrane phospholipids has significant effects. The ability of cholesterol to pack tightly with saturated fatty acyl chain groups of membrane phospholipids is critical for the formation of liquid-ordered rafts [10]. A physiological free cholesterol/phospholipid ratio in cellular membranes is necessary to maintain proper membrane fluidity and permeability.

In controls, we found free and esterified cholesterol in myelin and synaptosomes and small amounts of lanocholesterol in synaptosomes (Table 2). The dynamic equilibrium between free and esterified cholesterol in the brain is controlled by the activity of the respective sterol ester hydrolases. Free cholesterol was the most prominent component and ranged from 59% (in myelin) to 71% (in synaptosomes) of total cholesterol.

The higher content of free cholesterol in synaptosomes can probably be explained by the hypothesis about its local synthesis in synaptic terminals. There are data showing that cholesterol levels strongly influence the establishment and maintenance of synaptic connections and the basic synaptic processes and plasticity [11].

Glycolipids are important constituents of cells and their concentration is highest in the nervous system. Our results showed that gangliosides and cerebrosides were the two main glycolipid classes in controls and they accounted for 48% to 50% and for 50% to 53% of total glycolipids in myelin and synaptosomes, respectively (Table 3).

Synaptosomes contained higher amounts of gangliosides in comparison to myelin and these data suggest the specific role of gangliosides in synaptic transmission. Gangliosides are thought to be functional in memory formation too [12, 13].

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 [14].

It is well known that FFAs in the brain are normally present in small amounts and the brain FFAs pool is maintained at a low level in a state of dynamic equilibrium with membrane phospholipids. Among the individual FFAs, C16:0, C18:0 and C18:2 were the most prominent in myelin and synaptosomes in control rats (Table 4). Our study indicated that myelin is enriched with saturated fatty acids and therefore it is characterized by a high ratio of saturated to unsaturated fatty acids. Synaptosomes were enriched in C20:4. Most probably it is due to the biological role of C20:4 as a main precursor of prostaglandins, considered as modulators in synaptic processes. Synaptosomes are characterized by active ion transport across their membranes and the high unsaturation level increases the membrane permeability.

Our results confirmed that the ischemic process influences the phospholipid metabolism in the brain. In myelin, we found decreased levels of total phospholipids by 2.3% (Fig. 1). The different phospholipid classes were not equally affected. The concentration of PA, PI, SM and PE increased by 75.2%, 237.3%, 39.2% and 6.4%, respectively (Table 1).

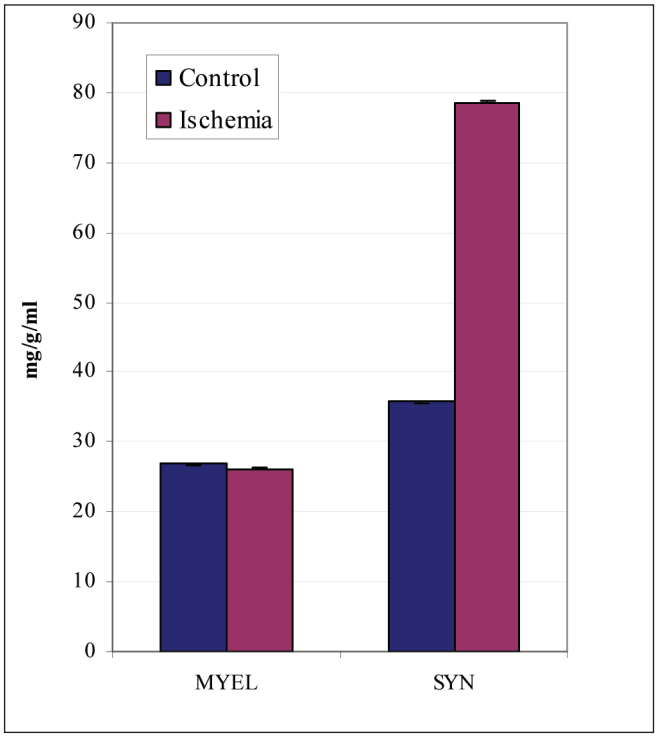

Fig.1 Changes of the total phospholipid content in myelin and synaptosomes after cerebral ischemia. p< 0.001

However, the content of PS and PC decreased by 10.6% and 29.3%, respectively. The inhomogeneous reduction of the various classes may be influenced by differences in their turnover. Another possible reason is the difference in the substrate specificity for each phospholipid or in the accessibility of phospholipase A2 to phospholipids due to a different distribution of each phospholipid in the membrane [15]. The increased susceptibility of membrane phospholipids to the action of specific phospholipases which leads to phospholipid degradation, might explain the decrease of the total phospholipid content in myelin after ischemia.

In synaptosomes the total phospholipids increased by 119.5% (Fig.1). Despite the high activity of phospholipases A2, C and D in synaptic membranes [16], it can be suggested that the increased content of total phospholipids in synaptosomes is due to a higher rate of synthesis during the recirculation period. This is supported by studies on the reversibility of lipid changes during reperfusion [17]. In synaptosomes we found increased content of all individual phospholipids except for PA whose concentration decreased by 64.7%. Numerous studies show that inositol-containing phospholipids are first degraded in ischemia followed by the release of diacylglycerides and their subsequent degradation to FFAs and glycerol [18]. The observed lower content of PA, which is a precursor for the synthesis of phospholipids, in the synaptosomal fraction allows us to suggest that it is due to an active synthesis of PI in the synaptosomal membranes, reflected by the nearly 10-fold increase of PI in synaptosomes from ischemic brain. The analysis also demonstrated increased concentration of LysP, PI, PS, PC and PE by 330.5%, 830%, 192.4%, 117% and 90.1%, respectively (Table 1).

The hydrolysis of membrane phospholipids disrupts the integrity of the membrane which can lead to the release of active cholesterol whose esterification can be stimulated by free fatty acids, and particularly by oleic acid. Considering that in cerebral ischemia there is a large amount of fatty acids released, it is feasible to expect high levels of esterified cholesterol. Esterification of free cholesterol renders it inaccessible for other metabolic modifications, such as demethylation, reduction, isomerisation of double bonds, hydroxylation [19].

We found an increase in both free and esterified cholesterol (Table 2) and as a result the total cholesterol estimates increased 50.4-fold and 13.8-fold in myelin and synaptosomes, respectively (Fig. 2). Free cholesterol made up 93% of the total cholesterol in myelin and 42% of total cholesterol in synaptosomes.

Our results showed that the firm and difficult to access ”sandwich-like” complexes between phospholipids, cholesterol and cerebroside in myelin, are susceptible to ischemic damage. These changes result in destruction of membrane integrity that could influence the transmission of nerve impulses. Some authors suggest a link between the high content of esterified cholesterol and the initial stage of demyelination.

The synaptosomes contained a significantly higher amount of esterified cholesterol compared with the myelin. A number of investigators report that cholesterol turnover is most intensive in synaptosomes. The high concentration of sterol esters in this fraction can be explained with a 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 and there is a striking correlation between the severity of tissue injury and the extent of cholesterol accumulation [20].

In the brains of rats subjected to cerebral ischemia, we found increased levels of total glycolipids (Fig. 3), gangliosides and cerebrosides (Table 3). The increase of total glycolipids was 1.8-fold and 2.3-fold in myelin and synaptosomes, 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 CNS injury by protection of membrane structure and function [21]. Another hypothesis

Brain fraction		Phosphatidic acid	Lysophospho-lipids	Phosphatidyl-inositol	Sphingo-myelin	Phosphatidyl-serine	Phosphatidyl-choline	Phosphatidyl-ethanolamine
Myel	Control	0.576±0.02	–	0.118±0.03	2.723±0.05	4.189±0.05	8.89±0.03	10.31±0.02
	Ischemia	1.009±0.09 p<0.001	–	0.398±0.07 p<0.001	3.789±0.07 p<0.001	3.747±0.08 p<0.001	6.285±0.06 p<0.001	10.973±0.09 p<0.001
Syn	Control	3.63±0.07	0.623±0.03	0.687±0.04	–	5.227±0.08	14.889±0.1	10.743±0.07
	Ischemia	1.281±0.04 p<0.001	2.059±0.07 p<0.001	6.389±0.06 p<0.001	0.817±0.05 p<0.001	15.284±0.07 p<0.001	32.312±0.07 p<0.001	20.423±0.09 p<0.001

Table 1. Changes of the phospholipid classes in myelin (Myel) and synaptosomes (Syn) after cerebral ischemia. Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

Brain fraction		Free cholesterol	Esterified cholesterol	Lano-cholesterol
Myel	Control	0.044±0.01	0.029±0.01	–
	Ischemia	3.454±0.18 p<0.001	0.274±0.03 p<0.001	–
Syn	Control	0.672±0.13	0.227±0.02	0.029±0.001
	Ischemia	5.357±0.17 p<0.001	7.458±0.56 p<0.001	–

Table 2. Changes of the cholesterol content in myelin and synaptosomes after cerebral ischemia. Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

Brain fraction		Gangliosides	Cerebrosides
Myel	Control	0.107±0.03	0.118±0.03
	Ischemia	0.093±0.004 p–	0.307±0.02 p<0.001
Syn	Control	0.227±0.04	0.23±0.04
	Ischemia	0.443±0.05 p<0.001	0.614±0.03 p<0.001

Table 3. Changes of the glycolipid classes in myelin and synaptosomes after cerebral ischemia. Values are expressed in mg/g dry lipid residue/ml.

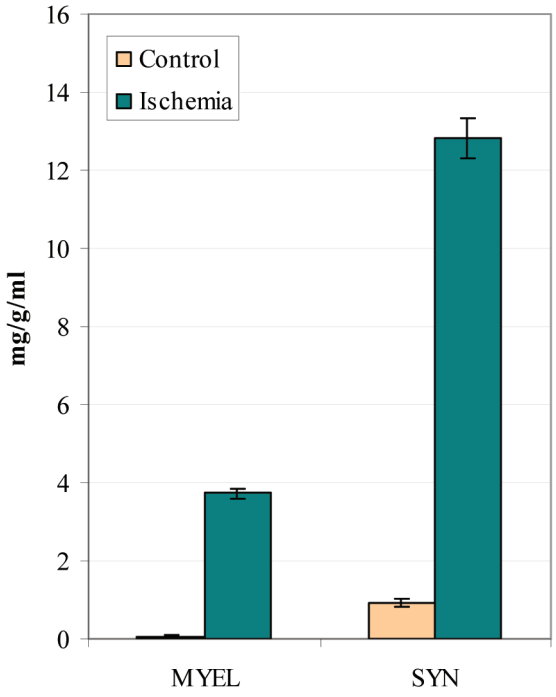

Fig. 2. Changes of the total cholesterol content in myelin and synaptosomes after cerebral ischemia. p<0.001

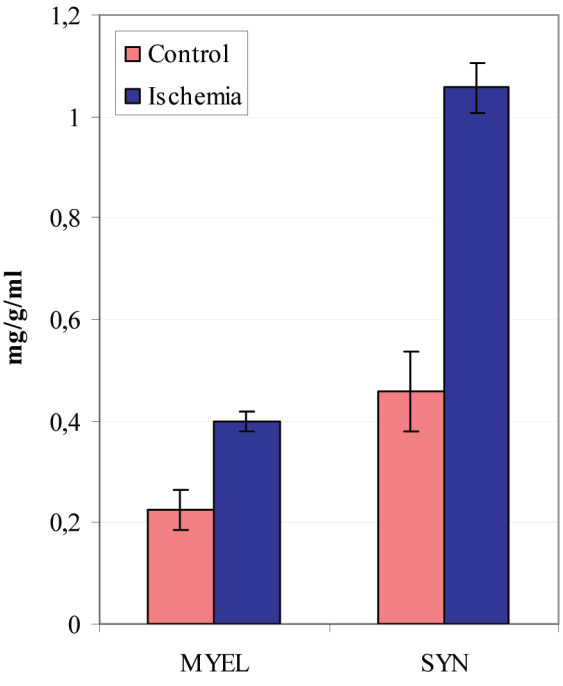

Fig. 3. Changes of the total glycolipid content in myelin and synaptosomes after cerebral ischemia. p<0.001

Brain fraction		$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{20:2}$	$C_{20:4}$
Myel	Control	0.02±0.001	0.287±0.03	–	13.541±0.8	–	0.95±0.02	0.581±0.01	–	0.002±0.001
	Ischemia	–	–	0.15±0.02	0.528±0.03 p<0.001	0.528±0.02	–	–	–	2.619±0.02 p<0.001
Syn	Control	0.26±0.01	0.512±0.02	–	0.348±0.04	–	0.801±0.06	0.308±0.01	–	4.22±0.07
	Ischemia	–	–	0.596±0.02	10.515±0.09 p<0.001	0.84±0.04	–	–	0.258±0.02	18.575±0.07 p<0.001

Table 4. Changes of the individual FFAs in myelin and synaptosomes after cerebral ischemia. Values are expressed in mg/g dry lipid residue/ml. A dash indicates trace amounts.

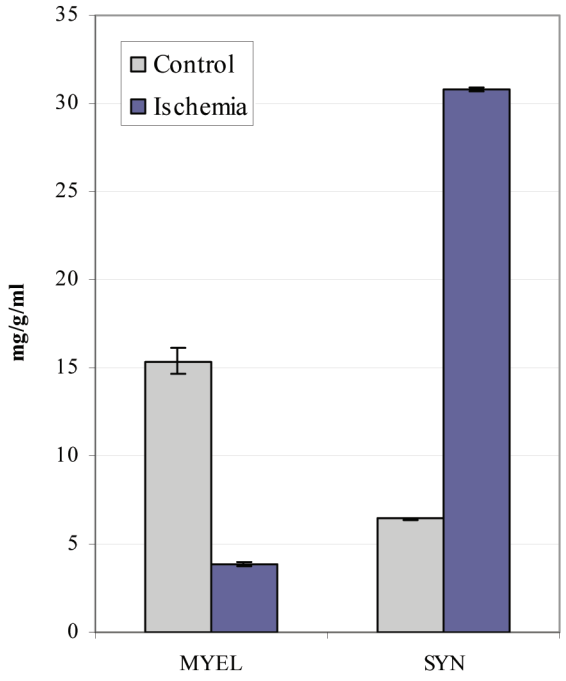

Fig. 4. Changes of the total FFAs content in myelin and synaptosomes after cerebral ischemia. p<0.001

supports 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 [14].

It is well known that FFAs are the first that undergo changes in different pathological states, including cerebral ischemia. We found a progressive increase of total FFAs in the synaptosomes - 4.8 times the control values (Fig. 4). Surprisingly, the myelin showed 4-fold decrease in total FFAs and it might be due to reesterification.

The major components of increased FFAs were C16:0, C18:0 and C20:4 acids (Table 4). It is considered that C18:0 and C20:4 are derived from inositol-containing phospholipids by the action of phospholipase C [22]. Released C20:4 may be metabolized by cyclooxygenases, lypoxygenases to form prostaglandins, leucotriens, and reactive oxygen species (ROS). Oxidative metabolism of C20:4 is considered to be a major source of ROS in ischemia, which may generate lipid peroxides and cytotoxic products like 4-hydroxynonenal, acrolein and malondialdehyde [23].

A notable observation was the accumulation of C16:1, C18:1, C20:2 and C22:6, which were absent in control rats. Probably they are derived from PE and PC by the action of phospholipase A2. Numerous studies show that the fatty acid composition of PE plus PC is more abundant in palmitic acid and docosahexaenoic acid than in stearic acid or arachidonic acid [18]. The high concentration of unsaturated FFAs after cerebral ischemia is probably due to their neuroprotective effect. It is reported that polyunsaturated FFAs (especially C20:4) block neuronal death by inhibiting glutamatergic transmission [24].

In conclusion, the results of the present study reveal that cerebral ischemia disrupts to a great extent the lipid metabolism in synaptosomes and myelin. The changes in membrane lipids are associated with impaired energy metabolism and most probably they are directed to improvement of the functional adaptive possibilities of the brain during crisis situations.

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