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Changes of Phospholipids and Their Fatty Acid Composition in Subcellurar Structures from Ageing Rat Brain

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The phospholipid composition, cholesterol and fatty acid content of subcellular fractions from ageing brain were studied. The phospholipid composition during ageing is characterized by specific phosphatidylcholine and phosphatidylethanolamine values. The content of cholesterol is also characteristic for the subcellular fractions. The fatty acid composition of phosphatidylcholine and phospatidylethano-lamine differed in the individual subcellular fractions. The changes of the lipids and cholesterol during ageing may affect the properties of the synaptic membranes and may result in hindrance of synaptic performance.

Key words: subcellular fractions, lipids, fatty acid composition, ageing brain

Introduction

The individual lipid classes play an important role in the processes of brain development and ageing and participate in the specialized functioning of the brain. There are data suggesting that certain members of the phospholipid, glycolipid and the neutral lipid classes are vital for the functional integrity and for the plasticity of the brain [4, 11, 13]. The lipids also play an inportant role in the transmission of information between nerve cells and other cell types (e.g. nerve, muscle, secretory cells). Lipids undergo specific changes during brain development [10]. In previous publications we described the changes in the lipids during early stages of brain development [4, 12]. Our results show that there are characteristic changes in individual lipid classes and their fatty acid composition (FAC). In the specialized literature the information on compartmentation of lipids in distinct subcellular organelles during the later stages of postnatal brain development is rather scarce. The nuclear fraction has the lowest lipid content, followed by the mitochondria and the cell membranes [9]. Additional information on this subject can be found in the papers treating the lipid content of fractions enriched with synaptosomes and growth cones [4, 10].

A significant reduction of membrane fluidity of mitochondria isolated from the brains of patients with Alzheimer's disease has been observed [7]. The results of S v e n n e r h o l m et al. [14], describing the changes of the weight and the compo-

sition of major membrane components of the human brain during the span of adult human life of Swedes are also of interest in this respect. These authors reported that there are changes of proteins and major lipids in subjects aged 20-100 years. The changes are specific with a manifested decrease of the phospholipids, cholesterol, sulfatides and gangliosides. An analysis of these changes could be useful in the search for ways to obviate certain pathological aspects accompanying the processes of brain ageing.

For this reason we studied the changes occurring in the subcellular fractions isolated from rat brain during ageing, comparing adult and ageing rats, aged 30 and 150 days respectively.

Material and Methods

Preparation of subcellular fractions

Subcellular fractions were isolated from the brains of Wistar rats aged 30 and 150 days. 10% homogenate was prepared in 0.32 M sucrose in Tris-HCl buffer, pH 7.4. The first pellet (P_1) obtained on centrifugation of total homogenate at 5000 × g for 5 min was resuspended in 10 cm^3 and was layered on a 2.0 M sucrose cushion. Centrifugation at 120 000 × g for 1 hour yielded the nuclear fraction. The supernatant (S_1) was centrifuged at 15 000 × g for 30 min. The resulting pellet (P_2) was resuspended in 20 cm^3 and was layered on a discontinous gradient, consisting of 20 cm^3 0.8 and 1.2 M sucrose steps. The gradients were spun in a swing-out rotor at 55 000 × g for 2 hours. The interphase 0.8-1.2 M sucrose contained synaptosmes and the pellet at the bottom - mitochondria. The supernatant (S_2) was centrifuged at 120 000 × g for 1 hour yielding a pellet containing endoplasmatic reticulum.

All fractions were diluted to 50 cm^3 with 0.32 M sucrose and subsequently pelleted at 90 000 × g for 30 min. The final pellets were diluted appropriately and analysed further.

Determination of phospholipids, cholesterol and fatty acid content (FAC) of phospholipids

Total phospholipids were separated on a Florasyl column by the method of R o u s e l et al. [8]. The phospholipids are designated as follows: phosphatidylcholine (PCh), phosphatidylethanolamine (PEA), phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL), sphyngomyelin (SM) and phosphatidic acid (PA) [1]. Cholesterol was determined according to M i l l e r et al. [6] and for determination of the FAC of phosphlipids we used the technique of K a t e s [5].

Results and Discussion

Phospholipid content of subcellular fractions

The mitochondrial fraction contains the highest amounts of CL. The synaptosomal fraction hardly contains CL, however, it exhibits the highest content of PI. The amount of PCh in mitochondria from 150 day old rats is 20% higher than that established in 30 day old rats, while PEA is 8% lower. There are characteristic changes in the synaptosomal fraction, whereby PCh in 150 day old rats was 8.6% lower, however, there is a significant increase of PI, amounting to 56% (Table 1).

Table 1. Amounts of phospholipids (%) of total lipid phosphorus

Phospholipid	Subcellular fraction							
	homogenate		nuclei		mitochondria		synaptosomes	
	150 days after birth	30 days after birth	150 days after birth	30 days after birth	150 days after birth	30 days after birth	150 days after birth	30 days after birth
Phosphatidyl-ethanolamine	38.3	39.4	38.6	36.9	30.0	33.0 $p<<0.05$	30.2	28.8
Phosphatidyl-choline	31.1	30.5	31.0	32.2	36.7	30.0 $p<<0.02$	47.1 $p<<0.05$	52.0
Phosphatidyl-serine	12.4	13.1	15.3	16.7	11.4	12.0	11.0	10.7
Phosphatidyl-inositol	4.4	3.6	3.3	3.0	2.3	2.1	7.8 $p<<0.01$	5.0
Cardiolipin	3.2	3.0	0.4	0.6	11.9	11.1	—	—
Sphyngo-myelin	8.6	8.4	9.8	9.2	6.4	6.7	2.9	3.1
Phosphatidic acid	2.0	2.0	1.6	1.4	2.3	2.1	1.0	1.4

Cholesterol content of subcellular fractions

Highest levels of cholesterol are found in the mitochondrial fraction, whereas the endoplasmatic reticulum exhibits the lowest ones. The synaptosomal fraction from 150 day old rats cotained 7.5 % lower cholesterol compared to 30 days old rats (Table 2).

Table 2. Cholesterol content (μg/mg dry lipid residue)

Subcellular fraction	Age (days after birth)	
	150	30
Homogenate	229.7 ± 11.9	237.0 ± 10.6
Nuclei	47.3 ± 0.6	46.6 ± 1.7
Mitochondria	98.3 ± 8.1	100.7 ± 7.3
Synaptosomes	60.3 ± 2.4	65.3 ± 1.2 $p<<0.02$
Endoplasmic reticulum	23.8 ± 5.5	25.1 ± 4.0

FAC of phospholipids

Analysis of the data summarised in Table 3 reveals that there are significant changes of the FAC only in PEA and PCh. The individual subcellular fractions are characterised by specific FAC, and changes of the per cent ratios of FAC of PEA and PCh. The nuclear fraction lacks the irreplaceable unique $C_{18:2}$, $C_{16:1}$ and the polyenic $C_{22:5}$. Mitochondrial PEA and PCh contain the full spectrum of FA. The synaptosomes prepared from 150 day old rats lack the polyenic long chain FAs with the exception of $C_{20:4}$ (arachidonic). One should note the substantial amount of $C_{18:2}$ in PEA.

Table 3. Content (%) of FA in PEA and PCh

Fatty acid	Age (days)	Subcellular fraction							
		Homogenate		Nuclei		Mitochondria		Synaptosomes	
		PEA	PCh	PEA	PCh	PEA	PCh	PEA	PCh
$C_{14:0}$	150	16.1	10.4	8.6	4.2	4.3	8.7	6.9	8.0
	30	16.0	10.7	—	4.0	5.0	9.1	4.0	6.6
$C_{16:00}$	150	28.3	24.5	20.5	20.0	16.4	16.7	20.3	10.7
	30	27.9	25.0	21.1	20.3	15.8	16.2	18.2	9.3
$C_{16:1}$	150	1.3	1.7	tr.	tr.	1.2	3.7	3.2	7.2
	30	1.5	1.7	—	—	1.2	3.6	1.3	3.1
$C_{18:0}$	150	30.3	28.4	25.1	33.6	39.0	35.4	38.4	42.6
	30	31.2	27.9	24.8	32.6	39.5	35.2	47.0	42.0
$C_{18:1}$	150	5.1	3.1	21.2	19.1	10.4	8.3	14.0	14.7
	30	5.0	3.0	20.2	20.5	10.5	8.2	11.3	10.1
$C_{18:2}$	150	0.2	0.9	—	—	2.2	4.5	10.5	2.3
	30	0.2	0.9	—	—	2.0	4.7	tr.	tr.
$C_{20:0}$	150	1.1	2.4	4.7	0.3	6.5	0.2	0.6	2.9
	30	1.3	2.0	5.3	0.5	6.5	0.2	1.0	3.6
$C_{20:4}$	150	8.3	9.3	10.4	12.3	7.4	11.6	6.1	11.6
	30	8.5	9.0	9.8	11.3	8.0	11.2	6.0	10.4
$C_{22:0}$	150	3.7	4.2	3.1	0.2	—	3.5	—	—
	30	3.3	4.5	3.1	0.2	—	—	3.5	2.7
$C_{22:5}$	150	1.2	3.0	—	—	1.5	3.4	—	—
	30	1.2	3.0	—	—	1.4	4.6	4.0	4.2
$C_{22:6}$	150	4.4	12.1	6.4	10.5	11.2	4.0	—	—
	30	3.7	12.3	6.7	10.1	11.1	7.0	3.7	8.0

Our results depict specific changes in the amounts of phospholipids, cholesterol and the FAC of individual phospholipids in subcellular fractions from ageing brain. It is well known that phospholipids participate in the control of the structure, permeability and regulation of enzyme activities of neuronal membranes [3] and cholesterol increases membrane stability and decreases mobility and permeability [2, 11].
The changes described above apparently reflect the activity of factors significant for the ageing process and for the development of age associated diseases. Further studies in this direction may help to reveal such factors and ways to influence or avoid their pathological effects.

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