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Phospholipid composition of pt rabbit and control rabbit brain

The skeleton of biological membranes is a phospholipid bilayer composed of a hydrophilic outer leaflet, a hydrophobic region and an inner hydrophilic leaflet. This lipid bilayer is characterized by functional asymmetry with respect to the distribution of the different phospholipids between the outer and inter leaflets (7). Neuronal membranes are highly specialized structures involved in receiving, processing, transporting and transmitting information. These functions are not only dependent on receptors, ion channels and enzymes but also on delicate balance in lipid composition of the membranes. Lipids provide the membranes with suitable stability, fluidity and permeability. The brain is characterized by a high content of neutral lipids, glycolipids, and phospholipids (12). Phospholipids contain relatively high concentrations of phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI). The brain has also the highest concentrations of proteins covalently modified with lipids, such as myristic and palmitic acid (8).

Phospholipids are fundamental cell membrane components with a high content of polyunsaturated fatty acids (PUFAs). It has been shown that diversity in the fatty acyl moieties of the phospholipid bilayer influences the biophysical properties of cell membranes. In neurons, synapses have the highest concentration of long PUFAs, especially docosahexaenoic acid and arachidonic acid (11).

The model of our study is a neurological sex-linked recessive mutation – rabbit pt (paralytic tremor), which is characterized by rhythmic body tremor and spastic paresis. Morphological studies showed a delayed irregular and defective myelination in the central nervous system (CNS). The mutation is characterized by transversion T>A in exon 2 of PLP-gene. The level of PLP-gene expression was extremely low in pt rabbit brain (5, 9).

In this study we analyzed the percentage content of phospholipids and unsaturated fatty acids in different areas of pt rabbit brain in comparison to control rabbit brain.

MATERIAL AND METHODS

Eight-week pt rabbits and eight-week New Zealand rabbits (control group) were used as experimental material. After decapitating the animals, the brain was isolated and the brain hemispheres, brainstem and cerebellum were prepared. The homogenization of tissues was carried out in 0.9% NaCl at +4°C. The 10% homogenate was used to determine phospholipids and fatty acids in pt rabbit brain and control rabbit brain.

Lipid extraction was performed according to Folch et al. (6). Phospholipids were analyzed by thin-layer chromatographic method on precoated silica gel G plates (E. Merck, Darmstadt, Germany) as previously described (14). Fatty acid composition of particular phospholipid classes was analyzed by gas chromatography (Perkin Elmer F 30 apparatus, Perkin Elmer Ltd. Beaconsfield, Bucks, England) as described in another paper. Phosphorus content was estimated according to Bartlett (1).

Statistical analyses were performed by Student's unpaired t-test. $P<0.05$ values were considered statistically significant.

RESULTS AND DISCUSSION

In the course of investigations of the physiological effects of polyunsaturated fatty acids (PUFAs), the oxidation of PUFAs in cell membranes has received considerable attention because of its possible contribution to the potential damage to biological systems. Therefore, the purpose of this work was to determine the proportion of phospholipids PE – phosphatidylethanolamine, PC – phosphatidylcholine, PS – phosphatidylserine, PI – phosphatidylinositol, PA – phosphatidic acid and Sph – sphingomyelin in different parts of pt rabbit brain and of control rabbit brain.

Table 1 and 2 show the percentage composition of phospholipids in brainstem, brain hemisphere and cerebellum of control rabbit brain. In brain hemispheres the levels of phospholipids were different in comparison with brainstem and cerebellum. The ratio PE/PC in brainstem and cerebellum was 1.3, in brain hemisphere it was 0.88.

Table 1. Phospholipid composition in different brain areas of control rabbit (% of total)

	Brainstem	Brain hemisphere	Cerebellum
	A	B	C
PE	35.1±3.5*	30.3±3.4**	35.3±4.0
PC	27.5±2.9*	35.5±4.1**	28.3±3.0
PS	17.7±1.9	16.5±1.8	17.5±2.0
PI	4.2±0.5*	1.6±0.3**	4.0±0.6
PA	0.4±0.1	0.3±0.1	0.3±0.1
Sph	14.1±1.5*	11.1±1.3**	13.6±1.6
Others	1.0±0.2*	5.1±0.6**	1.0±0.2

Mean values ± SD. Abbreviations used: PE – phosphatidylethanolamine, PC – phosphatidylcholine, PS – phosphatidylserine, PI – phosphatidylinositol, PA – phosphatidic acid, Sph – sphingomyelin. * – $p<0.05$ A vs. B, ** – $p<0.05$ B vs. C, *** – $p<0.05$ C vs. A

Table 2. Phospholipid composition in different brain areas of pt rabbit (% of total)

	Brainstem	Brain hemisphere	Cerebellum
	A	B	C
PE	34.6±3.8*	26.6±2.8**	33.4±3.6
PC	26.3±2.5*	30.0±3.1	27.1±2.9
PS	17.4±1.9*	13.2±1.5**	16.6±1.8
PI	4.0±0.5*	1.7±0.3**	4.0±0.5
PA	0.3±0.1	0.4±0.1	0.3±0.1
Spy	14.4±1.6*	11.5±1.3	12.6±1.4
Others	3.0±0.4*	16.6±1.8**	6.0±0.8***

Mean values ± SD. Abbreviations used: PE – phosphatidylethanolamine, PC – phosphatidylcholine, PS – phosphatidylserine, PI – phosphatidylinositol, PA – phosphatidic acid, Sph – sphingomyelin. *– $p<0.05$ A vs B, ** – $p<0.05$ B vs C, *** – $p<0.05$ C vs A

Söderberg et al. (13) discovered a characteristic ratio in different human brain areas, 1.4 in cerebellum and 0.8 in brain hemisphere. Cristensen Lou et al. (3) reported an increase in the relative content of PC and Sph at the expense of PE in brain tumors. They supposed that a high PC/PE ratio was indicative of a low degree of cellular differentiation and organization, because this ratio decreased during intrauterine life. Ledwożyw and Lutnicki (10) observed an increase in PI content in brain tumors, in comparison with the cortex. Guan et al. (8) have shown that PE/PC ratio decrease was 1.0 in frontal cortex, 0.89 in hippocampus and 1.32 in the white matter in the brain with Alzheimer Disease (AD). The major finding in the brains with AD was a 20%–30% decrease of both PE and PC in frontal cortex. No clear changes were observed in PS, PL and Sph in all three regions when control and AD samples were compared. Domańska-Janik et al. (5) showed that in pt rabbit brain all the myelin lipids are reduced during development. Myelin yield in pt rabbit brain was reduced to 20%–30% of control. The molar ratio of galactolipids to phospholipids was lower in pt rabbit.

In our study, the proportion of percentage phospholipid content did not differ significantly in mutant brainstem and cerebellum as compared to control. However, the PE, PC and PS content decreased in pt brain hemisphere in comparison to control. The content of PE, PC and PS in control rabbit brain was 82% and in pt rabbit brain it was 70%. The ratio PE/PC did not differ but the ratio of phospholipids to sphingolipids was lower in pt rabbit – 6.1, in control rabbit 7.4 (Table 1, 2). Our study confirmed the results of other authors (2, 5).

Interestingly, we have observed an increase in stearate (C 18:0) acid in phospholipids from three areas of pt rabbit brain (brainstem, brain hemisphere and cerebellum) and a decrease in fatty acids: oleate (C 18:0), arachidonate (C 20:0), docosahexaenoate (DHA, C 22:6) (Table 3, 4). There were no significant changes in the other fatty acid species examined. Söderberg et al. (13) analyzed the fatty acids in different regions of human brain in Alzheimer's disease. The abundance of the major monounsaturated fatty acid of PE, 18:1, is not significantly altered in Alzheimer's disease, but there is a substantial increase in the relative amounts of the saturated components 14:0, 16:0, and 18:0. This is parallelled by a decrease in the polyunsaturated fatty acid 20:4, 22:4 and 22:6. Wikiel et al. (15) have shown that percentage composition of phospholipids in 'pt' myelin was characterized by a lower proportion of acidic phospholipids (phosphatidic acid, phosphatidylserine, and polyphosphocnositides). The molar ratio of galactolipid to phospholipids is significantly lower in hypomyelinating mutants in the white matter of the adult pt rabbits (4).

Table 3. Fatty acids in different brain areas of control rabbit (% of total)

	Brainstem	Brain hemisphere	Cerebellum
	A	B	C
16 : 0 DMA	0.5±0.1*	1.8±0.2**	1.5±0.2***
16 : 0	19.2±1.6*	24.3±1.6**	21.8±1.7***
16 : 1 (n - 7)	3.4±0.2*	0.5±0.1**	2.1±0.2***
18 : 0 DMA	1.0±0.2*	1.4±0.1**	0.8±0.1***
18 : 0	16.6±1.5*	22.6±1.7**	19.2±1.2***
18 : 1 (n - 9)	33.0±1.9*	18.3±1.3**	25.5±1.6***
18 : 2 (n – 6)	0.3±0.1*	1.5±0.2**	1.0±0.2***
18 : 3 (n – 3)	0.6±0.1	0.7±0.1	0.8±0.1***
20 : 0	2.5±0.2	2.2±0.2	2.3±0.3
20 : 4 (n – 6)	5.0±0.4*	6.4±0.4**	5.4±0.4
22 : 0	3.4±0.4	3.5±0.4	3.3±0.3
22 : 6 (n – 6)	2.3±0.3*	10.1±0.8**	7.4±1.2***
Others	to 100	to 100	to 100

Mean values ± SD. DMA – dimethylacetal, * – $p<0.05$ A vs B, ** – $p<0.05$ B vs C, *** – $p<0.05$ C vs A

Table 4. Fatty acids in different brain areas of pt rabbit (% of total)

	Brainstem	Brain hemisphere	Cerebellum
	A	B	C
16 : 0 DMA	0.5±0.1*	1.7±0.2	1.6±0.2***
16 : 0	22.5±1.6*	27.3±1.7**	25.1±1.8***
16 : 1 (n - 7)	2.5±0.2*	0.5±0.1**	1.7±0.2***
18 : 0 DMA	1.2±0.2*	1.6±0.2**	0.7±0.1***
18 : 0	22.5±1.7*	27.3±2.2**	23.1±2.0
18 : 1 (n - 9)	22.4±1.5*	12.4±1.0**	17.6±1.6***
18 : 2 (n – 6)	0.4±0.1*	1.2±0.2	1.1±0.2***
18 : 3 (n – 3)	0.7±0.1	0.6±0.1	0.6±0.1
20 : 0	2.6±0.2	2.3±0.2	2.4±0.3
20 : 4 (n – 6)	3.2±0.2	3.1±0.3**	1.5±0.2***
22 : 0	3.8±0.3	3.4±0.4	3.5±0.3
22 : 6 (n – 6)	1.7±0.2*	4.3±0.4	4.2±0.4***
Others	to 100	to 100	to 100

Mean values ± SD. DMA – dimethylacetal, * – $p<0.05$ A vs B, ** – $p<0.05$ B vs C, *** – $p<0.05$ C vs A

This report demonstrated the degree of myelin maturation and supports the concept that myelin disease in the pt rabbit mutant is primarily a dysmyelinating process. The decrease in the content of polyunsaturateds acids and in the lower ratio of phospholipids to sphingolipids in pt rabbit observed by us, seems to support this hypothesis.

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SUMMARY

Phospholipids are fundamental cell membrane components with a high content of polyunsaturated fatty acids (PUFAs). The paralytic tremor (pt) rabbit, a neurological mutant, exhibits hypomyelination transmitted in X-linked recessive fashion. This rabbit mutant was used for regional phospholipid and fatty acid analyses in different brain structures. Our experiment showed that the percentage content of PE, PC, PS in brain hemisphere of pt rabbit is reduced in comparison to control rabbit. The ratio of phospholipids to sphingolipids was lower in pt rabbit – 6.1, and 7.4 in control rabbit. The ratio PE/PC did not differ. We have observed an increase in stearate acid in three regions of pt rabbit brain and a decrease in fatty acids: oleate, arachidonate, decosahexaenoate. It is suggested that the changes in phospholipid composition may play a role in structural and functional membrane perturbations in paralytic tremor rabbit.

Zawartość fosfolipidów w mózgu królika pt i królika zdrowego

Fosfolipidy są ważnym elementem błon komórkowych. Skład błon fosfolipidowych ma istotny wpływ na ich płynność, transport oraz funkcję i aktywność enzymów związanych z błonami. Obiektem naszych badań był królik z dziedziczną drżączką poraźną (*paralytic tremor pt*) – genetyczny mutant neurologiczny. Mutacja ta charakteryzuje się transwersją T>A w egzonie 2 genu PLP, a jej skutkiem jest opóźniona i niepełna mielinizacja oraz zmiany w strukturze osłonek mielinowych. Analiza procentowej zawartości fosfolipidów nie wykazała istotnych różnic w obrębie pnia i móżdżku w porównaniu z korą mózgu królika pt. W półkulach mózgowych wykazano zmniejszenie procentowej zawartości PE, PC i PS w porównaniu z królikiem kontrolnym. U królika zdrowego procentowa zawartość tych frakcji wynosi 82%, u królika pt 70%. Ponadto u mutanta neurologicznego obserwuje się wyższą zawartość kwasu stearynowego, natomiast obniżoną oleinowego, arachidonowego i dekozaheksaenowego. Można przypuszczać, że zmiany w strukturze fosfolipidów oraz składzie kwasów tłuszczowych są konsekwencją nieprawidłowej mielinogenezy, wynikającej z podstawowej mutacji genetycznej.