The results can be used to explain mechanisms of inhibition of phosphoacylglycerols and diacylglycerols from pyruvate by nicotinamide in the liver of db/db mice [15]

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SELECTIVE INCORPORATION OF DIETARY ω3 POLYUNSATURATED FATTY ACIDS INTO RAT CEREBELLAR PHOSPHOLIPIDS

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Modern views on the role of polyunsaturated fatty acids (PUFA) of the ω3 family, as essential dietary factors, are associated with their use by the body as plastic material for the synthesis of membrane structures [2, 12]. Whereas the essential nature of α-linoleic acid 18:3ω3 is still in the stage of discussion, the role of its metabolites — icosapentaenic acid 20:5ω3 as a regulator of thrombus formation has been confirmed by numerous investigations [10, 14]. The chief PUFA of the ω3 family, namely docosahexaenic acid 22:6ω3, is an important component of membranes of neurons and the retina, and it determines their fluidity [3]; its mandatory presence in synaptic membranes evidently plays a definite role in the transmission of nervous impulses [5]. The fatty acids (FA) of this family enter the body only by consumption of fish fat, other seafood, and certain vegetable oils [8, 13]. Brain tissues have the highest content of PUFA with predominance of 22:6ω3; the FA composition of the brain lipids, moreover, is maintained fairly constant irrespective of the composition of the fat consumed [6]. If the diet is deficient in fat or if it is excessive, no marked changes have been observed in the composition of FA of the brain phospholipids (PL) [15]. Dependence of the concentration of ω3 PUFA in brain tissues on the character of the dietary fat has been examined only sporadically [4].

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TABLE 1. Content of ω6 and ω3 PUFA in Sphingomyelins and Inositol Phosphatides of Rat Cerebellum, % (M ± m)

FA	Diet		
	C	With L	With I
18:2ω6	0,57±0,44	1,44±0,10***	2,0 ±0,1
20:4ω6	5,87±0,43	4,01±0,40**	7±0,
20:5ω3	0,19±0,01	0,20±0,02	,22±0
22:4ω6	0,45±0,03	0,33±0,02	0,45±0
22:5ω3	0,19±0,01	0,25±0	0,23±0
22:6ω3	1,51±0,15	1,97 0,17	1,86±0,13
PUFA ω6/ω3	3,70	2,3	2,7

Legend. Here and in Tables 2 and 3: *p < 0.05, **p < 0.01, ***p < 0.001 compared with control.

Considering the role of the blood–brain barrier in the regulation of the supply of essential nutrients to the brain, it was decided to investigate to what extent the inclusion of different sources of ω3 PUFA in the diet could influence the content of the acids of this family in the composition of PL of the rat cerebellum. It was decided to study the cerebellum, as the part of the brain on which diet has the greatest influence in the postnatal period.

EXPERIMENTAL METHOD

For 6 weeks, 75 growing male Wistar rats were kept on a fat-free diet in order to exhaust their endogenous PUFA reserves; the animals were then divided into three groups, and for 3 months they were given artificial dietary mixtures ad libitum. The sources of protein (20% by calorific value) and carbohydrates (55%) were casein and starch; all the diets contained essential vitamins and minerals. To create different ω6/ω3 PUFA ratios, linseed oil (L), fat of the Ivasi sardine (*Sardinops sagax melanoticta*) combined with lard 1:3 (I), and a mixture of sunflower oil with lard 1:3, to act as the control (C) were used. The values of the ω6/ω3 PUFA ratios in these diets were 0.27 (L), 0.5 (I), and 45.0 (C). The fraction of fat in the diets was 24%, and the content of linoleic acid 18:2ω6 was 1.3%. FA of the ω3 family in diet L were represented only by 18:3ω (55.4%), whereas in case I their total amounted to 21%, mainly 22:5 and 22:6ω3. The animals were decapitated under ether anesthesia. The cerebellum was removed and homogenized, and lipids were extracted from it by the method in [9]. Aliquots of lipid extract were applied to plates with "Merck" silica-gel, and the PL were fractionated in a chloroform:methanol:water (65:25:4) system into sphingomyelins + inositol phosphatides (SM + IP), phosphatidylcholines (PCh), and phosphatidylethanolamines (PE). The PL were identified against appropriate standards (Sigma Chemical Co., USA). Zones of PL were scraped off the plates and methylated with acetyl chloride by the method in [1]. The methyl esters of FA (MEFA) thus obtained were analyzed by gas–liquid chromatography (GLC) and gas chromatography mass spectrometry (GCMS).

Single samples of total cerebellar and cerebral lipids as a whole also were tested. GLC analysis was carried out on an "Intersmat" chromatograph on a column packed with 10% Silar 10C on chromosorb W/HW 100-120 mesh. Fractionation was carried out under isothermic conditions, with temperatures of column, vaporizer, and detector of 180, 220, and 240°C respectively; the carrier gas was nitrogen, with a rate of flow of 40 ml/min. GCMS-analysis was carried out on a "Finnigan 3200" apparatus with capillary column measuring 0.25 × 60 m (phase E-30, carrier gas helium, 1.2 ml/min). The quantitative composition of the FA-fractions of cerebellar PL according to the GLC data were expressed as percentages of the total FA; the significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

The results of GLC analysis (Tables 1-3) revealed a high content of ω6 and ω3 PUFA in all classes of PL, with predominance of these acids in PE. PUFA of the ω3 family were represented mainly by 22:6ω3, and its level in the L group was higher than in the control; the increase in the fraction of 22:6ω3 for group I was smaller. The content of this acid in the SM + IP fraction was 1.97, 1.86, and 1.51, for PC it was 4.11, 3.24, and 2.76, and for PE it was 11.79, 9.45, and 8.13% in groups L, I, and C respectively. Whereas in the case of the diet with I 22:6ω3 not only is assimilated directly by the brain but is also synthesized from

TABLE 2. Content of ω6 and ω3 PUFA in Phosphatidylcholines of Rat Cerebellum, % (M ± m)

FA	Diet		
	C	With L	With I
18:2ω6	0,27±0,01	0,64±0,02***	0,23±0,01*
20:4ω6	6,32±0,60	4,12±0,12**	2,45±0,09***
20:5ω3	0,04±0,01	0,04±0,01	0,02±0,01
22:4ω6	0,79±0,04	0,69±0,03	0,67±0,03
22:5ω3	0,03±0,01	0,07±0,02	0,02±0,01
22:6ω3	2,76±0,18	4,11±0,25***	3,24±0,19*
PUFA ω6/ω3	2,63	1,30	1,02

TABLE 3. Content of ω6 and ω3 PUFA in Phosphatidylethanolamines of Rat Cerebellum, % (M ± m)

FA	Diet		
	C	With L	With I
18:2ω6	0,44±0,05	0,87±0,07***	0,59±0,04*
20:4ω6	7,53±0,68	4,45±0,42**	5,23±0,51*
20:5ω3	0,15±0,02	0,11±0,01	0,09±0,01
22:4ω6	4,47±0,65	3,85±0,31	2,98±0,25*
22:5ω3	0,16±0,02	0,21±0,03	0,12±0,01
22:6ω3	8,13±0,75	11,79±0,95**	9,45±0,69*
PUFA ω6/ω3	1,47	0,93	0,95

precursor acids (20:5, 22:5 and, least of all, 8:3ω3), for the diet with L, introducing only 18:3ω3, the supply of 22:6 was derived entirely from endogenous biosynthesis. It can be concluded from the FA composition of the experimental diets (total ω3 PUFA was 55.4% for L and 21% for I) that the 22:6 fraction, as the chief representative of ω3 PUFA in rat cerebellar PL, depended not so much on the set of acids of the ω3 family in the diet as on the total content of ω3 PUFA in it. The cerebellar lipids contained virtually no 18:3, 20:5, and 22:5ω3 FA, which were consumed in large quantities with the experimental diets, suggesting rapid desaturation and elongation of these acids up to 22:6ω3 at the level of the blood–brain barrier; it is unlikely that these processes take place directly in the cerebellar tissues. This hypothetical mechanism of the utilization of 18:3ω3 by the brain is confirmed by data on incorporation of the main fraction of radioactivity into 22:6ω3 brain lipids 48 h after injection of ^{14}C-18:3ω3 into animals [7].

In all PL, during consumption of the experimental diets the arachidonic acid 20:4ω6 level was observed to fall. The concentration of 18:2ω6, which was increased compared with the control, and the content of acids 20:4 and 22:4ω6, which was simultaneously reduced, are proof of the inhibitory effect of FA of the ω3 family on biosynthesis of higher ω6 PUFA [11]. This inhibitory effect was most marked for the PC fraction, especially on a diet with I. Reduction of the 20:4ω6 fraction in cerebellar PL under the influence of dietary acids of the ω3 family was less marked than in results obtained for other tissues; meanwhile, unlike in other tissues, the level of 20:5 and 22:5ω3 acids remained virtually constant. During analysis of the ω6/ω3 PUFA ratio for the fractions and diets studied, a clear tendency was discovered for the level of ω3 PUFA in cerebellar PL to increase on the experimental diets, and this was accompanied by a simultaneous fall in the ω6 PUFA fraction. These results confirm that the alimentary factor has a direct influence on competitive inhibition of Δ5- and Δ6-desaturases, which are responsible for synthesis of tissue PUFA on the ω3 and ω6 families, and they enable this rule to be extended to brain PL.

The results of GLC analysis of MEFA isolated from total cerebellar lipids showed that the character of this change in the FA composition under the influence of different diets is similar to changes observed for the PL case. The same tendency also was observed when total lipids both from the cerebellum and from the brain as a whole were compared. Gas chromatography mass spectrometry data on the FA composition of total cerebellar lipids from rats of the experimental and control groups are particularly interesting for they reveal several unusual components. The widely represented dimethylacetals (DMA), are methylation products of the corresponding aldehydes — 16:0-, 18:0-, and 18:1-DMA (total 5-7%); 18:1-DMA, moreover, like oleic acid 18:1, is represented by two positional isomers. Trace amounts of 20:0- and 20:1-DMA

also were observed. The unusually high (up to 19%) DMA level in the PE fraction, whereas their concentration in other classes of PL is low, indicates that an essential part of rat cerebellar PE consists of plasmalogens. An appreciable quantity (up to 4%) of 2-hydroxy acids (2-OH-22:0, 2-OH-23:0, 2-OH-24:0, and 2-OH-24:1 acids) was discovered, with predominance of 2-hydroxylignoceric acid 2-OH-24:0 among them. An increased content of two positional isomers of icosenic acid and also the presence of high-molecular-weight FA with an odd number of carbon atoms — 23:0 and 23:1, and also 25:0 and 25:1 — were observed.

The investigation showed that diets both with L and with I had a marked influence on the PL composition of the rat cerebellum, proof of the essential role of ω3 PUFA in nutrition, and of its importance in the choice of source of ω3 PUFA in the diet.

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