Vol. 30, No. 6, August 1993 BIOCHEMISTRY *and* MOLECULAR BIOLOGY INTERNATIONAL
Pages 1115–1125

ALTERATION IN 5'-NUCLEOTIDASE ACTIVITIES AND COMPOSITION OF LIVER AND BRAIN MICROSOMES OF DEVELOPING RATS FED DIFFERENT DIETARY FATS.

A. Ammouche,[1,2] M. Clément [1] and J.M Bourre[1].

1 INSERM U 26, Hôpital Fernand Widal, 200 rue du Faubourg St Denis 75475 Paris Cedex 10 France
2 Institut National Agronomique (INA). Département de Technologie et de la Nutrition Humaine. Alger.

Received June 15, 1993
Received after revision, July 30, 1993

SUMMARY

Four groups of male weanling rats were fed during three months, diets different in the nature of fats and the activity of 5' nucleotidase and fatty acid composition of brain and liver microsomes were studied. Group A were fed a standard commercial diet, group B a fat free-diet and group C and D a fat free-diet, containing respectively 10 % of peanut-rapeseed oil and 10% of salmon oil. In brain and liver microsomes, 5'-nucleotidase activity increased throughout the development for all diets (except for the fat-free diet). Slight differences were found in rats fed the peanut-rapeseed oil diet compared to controls estimated at the same time. However, in animals fed the fish-oil diet, 5' nucleotidase had the highest activity in both brain and liver microsomes. Marked changes occured in the fatty acid patterns of brain and liver microsomes among the various groups. The greatest alterations were found in the liver microsomes. In brain and liver microsomal membranes the fat-free diet induced an increase in monounsaturated fatty acids, an synthesis of eicosatrienoic acid, and a decrease in (n-6) and (n-3) polyunsaturated fatty acids. Animals fed a peanut-rapeseed oil and control diet showed similar fatty acid patterns in liver and brain microsomes. However,when rats were fed a fish-oil diet, the liver microsomal membranes were highly enriched in eicosapentaenoic and docosahexaenoic acids, and simultaneously there was a decrease in arachidonic acid. These results suggest that manipulation of the lipid environment influences 5'-nucleotidase activity by the interaction of the enzyme with specific membrane lipids

INTRODUCTION

Many studies published the last few years have described the effects of dietary lipids on human and animal body lipids (1-5) as well as their role in prevention of cardiovascular diseases and their effects on blood levels of cholesterol, triacylglycerol and lipoproteins (6). These studies have also investigated the relationship between the lipid composition of biological membranes, particulary in polyunsaturated fatty acids, and the proteins that determine the functional properties of membrane-bound enzymes such as 5'-nucleotidase (E.C.3.1.3.5, 5'ribonucleotide phosphohydrolase). This enzyme, widely distributed in mammalian tissues, is an ectoenzyme

1039–9712/93/121115–11$05.00/0

attached to the exterior of plasma membranes through a phosphatidylinositol glycan (7). It offers a potentially interesting model for studying the interactions between a membrane-bound enzyme and its lipid environment (8). 5'-Nucleotidase is thought to play an important role in several cells and tissues due to its key position in the breackdown of purine and pyrimidine nucleotides to their corresponding nucleosides (9). Its biological function is not completely understood. It has been proposed that 5'-nucleotidase provides nucleosides that are subsequently transported through the plasma membrane via a specialized carrier system (10). Adenosine, produced by the enzyme, can also act as a local hormone (11). In animals, including humans, provision of essential fatty acids in the diet leads to significant changes in the unsaturated fatty acid composition and fluidity of mammalian cell membranes (12,13). These changes are now being recognized as functionally important as the specific role of subcellular membrane lipids in modulating membrane function becomes more clear (14,15). This is particulary so with regard to the effects of dietary lipids on the physical properties of membrane lipids and the effect they have on the functioning of the various membrane enzymes associated with many physiological processes (16). Recent observations in our laboratory (17-20) have shown that the activity of 5'-nucleotidase decreased by 30 % in total brain, but not in myelin or in nerve endings of animals fed a (n-3) deficient diet as compared to those fed a "soy-bean" diet. Previously, Chandrasekhara and Ananth Narayan (21) reported a lower 5'-Nucleotidase activity in liver plasma membranes from essential-fatty-acid-deficient rats fed a purified diet. Awad and Chattopadhyay (22) reported lower 5'-nucleotidase activity in cardiac sarcolemma from rats fed a coconut oil diet compared with rats fed a sunflower oil diet. Flier and al (23) have observed that the specific activity of 5'-nucleotidase was significantly enhanced in membranes from rats fed menhaden oil. Although it is well known that dietary lipid manipulation can affect the lipid composition of biological membranes, and in turn, affect the biophysical characteristics of the membrane and the activities of certain membrane-associated enzymes, there is little knowledge on the rate of alteration of this enzyme at different ages. For this reason, the present study was initiated to determine the changes in brain and liver microsome fatty acid composition and the alteration over time in the membrane-bound 5'-nucleotidase activities following changes in dietary lipid intake and age of rats.

MATERIALS AND METHODS

Animals and diets

Experiments were performed on one hundred male weanling rats of the Sprague-Dawley strain weighing 40 ± 5 g purchased from Iffa Credo, l'Arbresle, (France). Rats were divided in four groups of 25 animals each and maintained under standardized conditions of temperature (22°C ± 1°C) and relative humidity (70%) with a 12-hour light-dark cycle. The animals were fed ad libitum and had free access to water. Group A received a standard laboratory diet, group B a fat-free diet, group C a fat-free diet supplemented with 10 % peanut-rapeseed oil (48-52 %), and group D a fat-free diet supplemented with 10 % fish-oil (salmon-oil). Fish-oil was supplemented with 100 mg alpha-tocopherol per 100 g oil. Diets were stored in the dark at 4°C. The composition of the

fat-free diet in weight percent was casein 22.5, glucose 63.5, cellulose 6, mineral mixture 7, and vitamin mixture 1. Experimental diets were prepared by adding 10g of either peanut-rapeseed oil or salmon oil to 90g of fat-free basal diet. Fatty acid composition of the diets are presented in table 1.

Preparation of microsomes

At various ages (21,40,60,80,100 and 120 days), animals were killed by decapitation without anaesthesia. Liver and brain were quickly excised, and washed with buffer solution (0.1 M Tris HCl, 0.32 M sucrose, 0.9 % NaCl pH 7.4), suspended in the same buffer solution at 25 and 20 % respectively and homogenized in a Potter homogenizer. The homogenates were centrifuged for 5 min at 8 000 g then the supernatants were centrifuged for 30 min at 16 000 g. The resulting supernatants were centrifuged for 60 min at 105 000 g.and microsomal pellets were resuspended in the same buffer to obtain about 2 mg of proteins /ml.

Assay of 5'-Nucleotidase

the activity of 5'-nucleotidase was assayed by measuring the release of inorganic phosphate from 5'AMP according to the method of Lindberg et al (24) with minor modifications reported by Cammer et al (25). The reaction was started by adding brain or liver microsomal suspensions (approx. 0.1 mg prot.) to the reaction mixture of 0.2 ml. The tubes were incubated at 37°C for 20 min in triplicate for each time point, then the reaction was stopped by adding 0.3 ml of 2.5 M H2SO4. Values were calculated on the basis of specific activity (nmol of phosphorus liberated per mg protein per min). Protein concentration was determined by the method of Lowry et al (26), using bovine serum albumin as a standard.

Lipid analysis

Lipids were extracted from aliquots of microsomes from each group according to Folch et al (27). Lipids were transesterified by treatment with methanol / cyclohexane / acetylchloride for 60 mn at 100° C according to the method of Lepage and Roy (28). The methyl esters were analyzed on a Delsi gas chromatograph equipped with a flame ionization detector and a silica capillary column (length 30 m, internal diameter 0.32 mm, stationary phase omegawax Supelco, France). Helium was used as the carrier gas. The oven, injector and detector temperatures were maintained respectively at 200, 230, and 250 °C. Identification of the peaks was performed by comparison with authentic commercial standards and with mixtures of known fatty acid composition. Areas were calculated with a Merck-Hitachi 2 500 integrator, and fatty acid concentrations were reported as percent of total fatty acid content. The double bond index was calculated from the sum of the percentages of each unsaturated fatty acid times the number of double bonds in that acid.

Statistical analysis

All data are presented as means ± S.D. Statistical comparison of means was made using of variance (ANOVA), and the effect of individual diets was compared for statistical significance ($p<0.05$) using the unpaired Student's t test.

RESULTS

Lipid composition of microsomal membranes

The fatty acid profiles of liver microsomes from rats fed various diets are presented in table 2. Minor differences were observed between control group and Peanut-rapeseed oil group. In

Table 1.
Fatty acid composition of the experimental diets.
Values represent the average obtained from at least three separate extractions and analyses of the respective diets and are relative amounts, expressed as a percentage of the total identified fatty acids. DBI, double bond index. S, saturated fatty acids.

Fatty Acid (%)	Diets		
	Control	Peanut-Rapeseed oil	Fish oil
14:0	1.08	0.06	7.48
16:0	-	0.02	0.6
17:0	-	0.06	0.88
18:0	11.63	2.32	2.76
20:0	0.39	0.84	-
22:0	-	1.51	3.32
Σ Saturated	13.10	4.81	15.04
16:1 n-9	0.27	0.21	11.81
16:1 n-7	1.34	-	1.18
18:1 n-9	42.36	52.22	15.90
18:1 n-7	2.29	-	4.43
20:1 n-9	0.92	3.12	8.80
Σ MUFA	47.18	55.55	42.12
18:2 n-6	16.52	27.10	2.07
18:3 n-6	0.15	0.02	-
20:4 n-6	-	-	0.65
Σ PUFA (n-6)	16.67	27.12	2.72
18:3 n-3	1.29	3.00	0.65
18:4 n-3	-	-	1.91
20:5 n-3	-	-	10.16
22:5 n-3	-	-	2.41
22:6 n-3	-	0.55	9.63
Σ PUFA(n-3)	1.29	3.55	24.76
n-6 /n-3	12.92	7.63	0.1
DBI/S	6.45	25.38	11.77

Table 2.
Fatty acids composition of liver microsomes of rats fed various dietary fats for a period of 3 months.Results are expressed as % of total fatty acids. Values (means ± S.D., n = 6) not bearing the same superscript letter are significantly different at $p<0.05$. If no superscript appears, values are not significantly different. tr: trace. DBI, double bond index. S, saturated fatty acids.

Fatty Acid (%)	Diets			
	Control	Fat free	Peanut Rapeseed oil	Fish oil
14:0	0.50 ± 0.01	0.44 ± 0.09	0.32 ± 0.02	0.55 ± 0.07
16:0	23.10 ± 0.66	20.85 ± 0.11	21.37 ± 0.30	24.93 ± 0.15
17:0	0.32 ± 0.05^{a}	0.32 ± 0.09^{a}	0.70 ± 0.10^{b}	0.50 ± 0.04^{ab}
18:0	18.60 ± 0.17	16.10 ± 0.10	18.94 ± 0.25	19.44 ± 0.70
20:0	0.23 ± 0.01	0.37 ± 0.02	tr	tr
Σ SFA	42.75 ± 0.90	38.08 ± 0.41	41.33 ± 0.67	45.42 ± 0.96
16:1 n-9	0.40 ± 0.06^{a}	2.33 ± 0.28^{b}	0.64 ± 0.08^{a}	0.31 ± 0.01^{a}
16:1 n-7	3.88 ± 0.43^{a}	7.80 ± 0.55^{b}	2.21 ± 0.11^{c}	3.35 ± 0.07^{ac}
18:1 n-9	13.20 ± 0.24^{a}	20.50 ± 0.91^{b}	13.10 ± 0.20^{a}	10.54 ± 0.40^{c}
18:1n-7	4.77 ± 0.18^{a}	8.70 ± 0.34^{b}	3.51 ± 0.14^{ac}	2.47 ± 0.11^{c}
20:1n-9	0.30 ± 0.05	0.33 ± 0.07	0.45 ± 0.02	0.34 ± 0.05
20:1 n-7	0.10 ± 0.02^{a}	0.40 ± 0.01^{b}	0.22 ± 0.01^{c}	0.32 ± 0.01^{d}
Σ MUFA	22.65 ± 0.98a	40.06 ± 2.16^{b}	20.13 ± 0.56^{a}	17.33 ± 0.65^{c}
18:2 n-6	8.30 ± 0.50^{a}	1.04 ± 0.06^{b}	11.30 ± 0.80^{c}	2.90 ± 0.05^{bd}
18:3 n-6	-	-	0.30 ± 0.01	0.15 ± 0.01
20:3 n-6	1.00 ± 0.02	0.30 ± 0.02	1.20 ± 0.04	0.90 ± 0.01
20:4 n-6	19.40 ± 0.13^{a}	5.94 ± 0.10^{b}	20.15 ± 0.50^{a}	12.91 ± 0.12^{c}
22:4 n-6	0.32 ± 0.02	-	0.34 ± 0.03	0.30 ± 0.02
22:5 n-6	0.80 ± 0.01^{a}	1.10 ± 0.01^{b}	0.40 ± 0.02^{c}	0.35 ± 0.01^{cd}
Σ n-6	29.82 ± 0.23^{a}	8.38 ± 0.19^{b}	33.39 ± 0.67^{c}	17.36 ± 0.21
20:5 n-3	-	-	0.90 ± 0.01	6.90 ± 0.02
22:5 n-3	0.53 ± 0.01	-	0.62 ± 0.01	1.90 ± 0.02
22:6 n-3	5.00 ± 0.01^{a}	1.08 ± 0.03^{b}	5.11 ± 0.03^{a}	12.02 ± 0.01^{c}
Σ n-3	5.53 ± 0.02^{a}	1.08 ± 0.03^{b}	5.73 ± 0 .04^{c}	20.82 ± 0.05^{d}
20:3 n-9	-	14.50 ± 0.20	-	-
Σ PUFA	35.35 ± 0.25^{a}	23.96 ± 0.24^{b}	39.12 ± 0.71^{c}	38.18 ± 0.26^{cd}
n-6/n-3	5.39 ± 0.02^{a}	7.75 ± 0.02^{b}	5.82 ± 0.01^{c}	0.83 ± 0.03^{d}
DBI/S	3.71 ± 0.01	3.20 ± 0.10	4.02 ± 0.03	4.30 ± 0.02
18:1/18:0	0.70 ± 0.03^{a}	1.27 ± 0.01^{b}	0.69 ± 0.03^{a}	0.54 ± 0.01^{c}
20:3 n-9/20:4 n-6	-	2.44 ± 0.02	-	-
20:3 n-6/18.2 n-6	0.12 ± 0.02^{a}	0.32 ± 0.01^{b}	0.11 ± 0.04^{a}	0.31 ± 0.05^{bc}
20:4 n-6/20:3 n-6	19.40 ± 0.52	19.66 ± 1.12	16.75 ± 1.35	14.33 ± 2.30

contrast marked changes were obtained in the liver microsomes from rats fed fat free diet or fish oil diet. Fat free diet induced in the liver microsomes an increase in monounsaturated fatty acids (MUFA), an active synthesis of 5,8,11,eicosotrienoic acid and a concomitant decrease in (n-6) and (n-3) polyunsaturated fatty acids (PUFA) as compared to the control. Higher ratios of C20:3(n-9)/C20:4(n-6), C18:1(n-9)/C18:0 (which are commonly used as an index of EFA deficiency) and C20:3(n-6)/C18:2(n-6) indicate a delta 9 and delta 6 desaturase stimulation (29). The double bond index/saturated fatty acid ratio was not significantly different from the control group. In animals fed the fish-oil diet, there was a general increase of the content of (n-3) fatty acids in liver microsomes compared with the control group. Linoleic and arachidonic acids, on other hand, decrease markedly, but the latter much more in liver than in brain. The high levels of docosahexaenoic and eicosapentaenoic acids evidently depress the elongation of the (n-6) series of fatty acids (8). Lower ratios of C20:3(n-6)/C18:2(n-6) and C20:4(n-6)/C20:3(n-6) could indicate that delta 6 and delta 5 desaturase enzymes were inhibited. The double bond index/saturated fatty acid ratio was similar to that of the control animals, but the (n-6)/(n-3) ratio naturally decreases profondly. However, brain microsomal fatty acid composition was only slightly affected by dietary lipid intake (Table 3)

5'-Nucleotidase activity

The activities of 5'-nucleotidase in rat at different times after feeding the various diets are shown in Fig. 1 (A and B). It can be seen that the specific activity of brain and liver microsome 5'-nucleotidase increased throughout development from 21 to 120 days for all diets (except for the fat free-diet). Thus, the specific activity of 5'nucleotidase in both brain and liver microsomes at 120 days was about 1.2 fold that at 21 days in rats fed the fat-free diet. This represented a reduction in specific activity of 15 and 30 % at 120 days in brain and liver microsomes, respectively, compared with the results for control diet at the same time. But no appreciable differences were found in rats fed a peanut-rapeseed oil diet compared with the control group. However, in animals given the fish-oil diet, the specific activity of 5'nucleotidase at 120 days was about 2-fold that at 21 days in both brain and liver microsomes. This represented an increased of 26 % and 40 % at 120 days in brain and liver microsomes, respectively, compared with the control diet.

DISCUSSION

It is clear from the experiments described in this paper that the fatty acid composition of the diet has a marked influence on the fatty acid composition and activity of 5'-nucleotidase particulary in liver microsomal membranes. The brain tends to maintain its fatty acid composition to a much greater extent than liver. It is, of course, very probable that this reflects the "protective" effect of the blood-brain barrier, although dietary polyenoic acids (particulary arachidonic and

Table 3
Fatty acid composition of brain microsomes of rats fed various dietary fats for a period of 3 months. Results are expressed as % of total fatty acids. Values (means ± S.D., n = 6) not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not significantly different. DBI, double bond index. S, saturated fatty acids. Tr, trace.

Fatty Acid (%)	Diets			
	Control	Fat free	Peanut Rapeseed oil	Fish oil
14:0	0.22 ± 0.02	0.24 ± 0.01	0.31 ± 0.04	0.40 ± 0.09
16:0	25.52 ± 0.3	25.59 ± 0.58	27.59 ± 0.69	25.36 ± 048
17:0	0.30 ± 0.01^{a}	0.28 ± 0.03^{a}	0.40 ± 0.01^{a}	0.77 ± 0.19^{b}
18:0	20.86 ± 0.11	20.72 ± 0.30	21.95 ± 0.48	21.25 ± 0.56
20:0	0.71 ± 0.05	0.50 ± 0.01	0.87 ± 0.05	1.23 ± 0.07
22:0	0.11 ± 0.00	0.16 ± 0.02	tr	tr
24:0	0.22 ± 0.01	tr	tr	0.10 ± 0.00
Σ SFA	47.94 ± 0.50	47.19 ± 0.95	51.12 ± 1.26	49.11 ± 1.39
16:1 n-9	0.24 ± 0.01	0.29 ± 0.09	0.22 ± 0.01	0.21 ± 0.03
16:1 n-7	0.61 ± 0.02^{a}	0.81 ± 0.01^{a}	0.79 ± 0.01^{a}	1.35 ± 0.20^{b}
18:1 n-9	14.28 ± 0.90	15.30 ± 0.74	15.13 ± 0.22	14.18 ± 0.35
18:1 n-7	4.42 ± 0.20	5.10 ± 0.55	4.83 ± 0.41	4.52 ± 0.20
20:1 n-9	0.54 ± 0.02	0.62 ± 0.08	0.67 ± 0.10	0.60 ± 0.09
20:1 n-7	0.25 ± 0.01	0.42 ± 0.04	0.46 ± 0.02	0.34 ± 0.01
22:1 n-9	tr	0.07 ± 0.01	tr	tr
Σ MUFA	20.34 ± 1.16	22.61 ± 1.52	22.10 ± 0.77	21.20 ± 0.88
18:2 n-6	0.86 ± 0.24^{a}	0.22 ± 0.01^{b}	0.87 ± 0.07^{a}	0.54 ± 0.06^{ab}
20:3 n-6	0.32 ± 0.01	0.22 ± 0.02	0.32 ± 0.05	0.32 ± 0.05
20:4 n-6	13.92 ± 0.62	12.25 ± 0.71	11.73 ± 0.94	11.85 ± 0.96
22:4 n-6	2.61 ± 0.06	2.15 ± 0.09	2.62 ± 0.51	1.75 ± 0.14
22:5 n-6	1.00 ± 0.12^{a}	1.86 ± 0.05^{b}	0.38 ± 0.09^{c}	0.51 ± 0.01^{cd}
Σ (n-6)	18.71 ± 1.59	16.70 ± 0.88	15.92 ± 1.64	14.97 ± 1.22
22:5 n-3	0.14 ± 0.02^{a}	0.20 ± 0.01^{b}	0.16 ± 0.01^{ab}	0.52 ± 0.01^{c}
22:6 n-3	13.92 ± 0.67	12.56 ± 0.47	11.76 ± 0.74	15.87 ± 0.85
Σ (n-3)	14.06 ± 0.69	12.76 ± 0.48	11.92 ± 0.75	16.39 ± 0.86
20:3 n-9	-	1.47 ± 0.02	-	-
Σ PUFA)	32.77 ± 2.28	30.93 ± 1.38	27.84 ± 2.39	31.36 ± 2.08
n-6/n-3	1.33 ± 0.02	1.30 ± 0.01	1.33 ± 0.03	0.91 ± 0.01
DBI/S	3.80 ± 0.34	3.62 ± 0.50	3.06 ± 0.24	3.71 ± 0.20
18:1/18:0	0.68 ± 0.02	0.73 ± 0.01	0.68 ± 0.01	0.66 ± 0.03
20:3 n-9/20:4 n-6	-	0.12 ± 0.01	-	-
20:3 n-6/18:2 n-6	0.37 ± 0.01^{a}	1.00 ± 0.00^{b}	0.36 ± 0.01^{a}	0.59 ±0.01^{c}
20:4 n-6/20:3 n-6	43.50 ± 0.02	55.68 ± 0.05	36.75 ± 0.01	37.03 ± 0.02

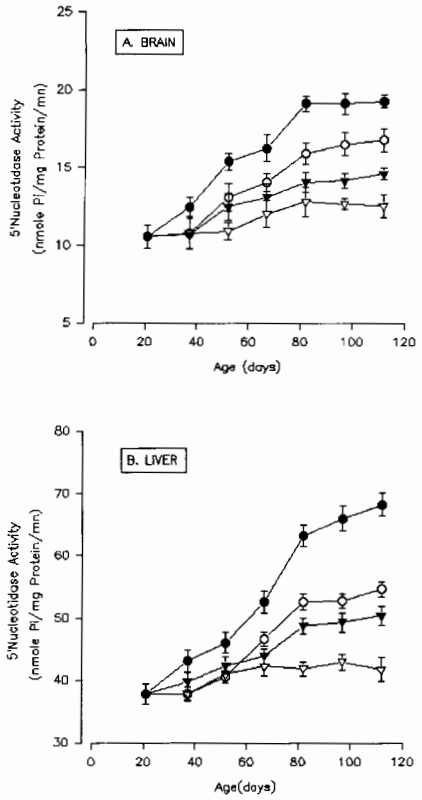

Figure 1.
Specific activity of 5' nucleotidase in microsomes of rats fed various diets at different ages. Values are means ± SD of 4 animals. Experiments were done in triplicate.
A. Brain
B. Liver.

● Fish-oil diet ▼ Control diet

○ Peanut-rapeseed oil diet ▽ Fat free diet

docosahexaenoic acids) are incorpored into brain phospholipids of rats (30). In order to interpret the enhancement of 5'-nucleotidase activity in brain microsomes, where there was no significant change in fatty acid composition, we shall suppose different possibilities of lipid influence on the enzyme activity. Dietary lipids can affect chemical and physical properties of membranes. Positional distribution and composition of fatty acids in the membrane is subject to intrinsic control. These intrinsic mechanisms theoretically could provide and maintain optimal membrane composition despite fluctuations in dietary lipid. Moreover, the activity of 5'-nucleotidase can be modulated by particular phospholipids, and may change also as a consequence of changes of metabolic rates. Feeding fats of different fatty acid composition does not appear to alter the ratio

of unsaturated-to-saturated fatty acids, suggesting that membranes generally display a considerable degree of homeostasis with respect to this parameter. This homeostasis suggests an important role for membrane lipids in regulating membrane and cellular functions. The decrease in 5'-nucleotidase activity elicited by a fat-free diet may be due to the diminished polyunsaturated fatty acid content of microsomal membranes and lead to increased synthesis of monounsaturated fatty ' acids to maintain the appropriate "fluidity" of membranes and the proper unsaturated/saturated fatty acid ratio. Moreover, alterations in pituitary function have been reported in essential fatty acid deficiency (31). Consequently, the decreased 5'-nucleotidase activity in the deficient membranes might result from altered pituitary function, perhaps in response to altered prostaglandin production, as well as from changes in membrane fatty acid composition. In early studies, investigators found that EFA-dependent decreases in (n-6) PUFA led to a 25 % to 50 % reduction in the specific activities of 5'-nucleotidase in liver (32) and cardiac (33) membranes. Bernsohn and Spitz (34) when they found that the significant (>60 %) reduction in 5'-nucleotidase activity in brain membranes from rats fed a fat-free diet for 4 months could be reversed by linolenic but not linoleic acid feeding. Consistent with this, Bourre et al (35), recently reported that diet-induced decreases in (n-3) PUFA (e.g., diets containing sunflower, peanut, soya, or rapeseed-oil) resulted in a 20 % reduction in enzyme activity in rat neural membranes. On other hand the increase in 5'-nucleotidase activity induced by a fish-oil diet could be due to changes in the membrane fluidity or to the specific effect of (n-3) fatty acids on the enzyme itself (36-38). Specific interactions between (n-3) PUFA and particular domains of proteins may affect catalytic properties of the enzyme, or localized changes in membranes may affect enzyme-substrate interactions. Johannson, Smith and Metcalfe (39) suggested that the optimal protein conformation for enzyme activity is maintained by lipids with chain lenghs which match the dimensions of the hydrophobic surface of the transbilayer part of the protein structure, leaving the polar head groups appropriately apposed to the more polar parts of the protein structure at the surface of the bilayer.

From these results, it appears that dietary fats can influence the activity of 5'-nucleotidase. The implications of increased 5'-nucleotidase activity following fish-oil ingestion are of potential significance. A higher 5'-nucleotidase activity, by releasing adenosine, a vasodilating agent (40), could increase blood flow to the heart. Adenosine also inhibits platelet aggregation induced by ADP in a competetive manner (11) and this inhibition could help in reducing thrombosis. Yannarell and Aronson (41) have suggested that 5'-nucleotidase may be involved in defense mechanisms protecting cells from foreign RNA. Adenosine can also depress the release of acetylcholine and other neurotransmitters, adversely affecting neuromuscular functions (42). Furthermore, the possibility that 5'-nucleotidase might be involved in triggering cell growth and differenciation has been suggested (43) and this may have implications in atherogenesis (44). In conclusion, the results of the present study suggest that the impact of dietary fats on the activity of 5'-nucleotidase can be mediated though factors such as fatty acyl composition of the membrane lipids and physical properties of the membrane.

ACKNOWLEDGMENTS

This work was supported by INSERM (Institut National de la Santé, et de la Recherche Médicale). The authors are grateful to Alan Strickland for English assistance.

REFERENCES

1. Purvis, J.M., Clandini, M.T and Hacker, R.R. (1973) Comp.Biochem. Biophys. 76B, 199-204.
2. Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta. 779, 89-137.
3. Conroy, D.M., Stubbs, C.D., Belin, J., Pryor, C.L. and Smith, A.D. (1986) Biochim. Biophys. Acta. 961, 457-462.
4. Brasitus, T.A., Davidson, N.O. and Schachter, O. (1985) Biochim. Biophys. Acta. 812, 960-971.
5. Spector, A.A. and Yorek, M.A (1985) J. Lipid. Res. 26, 1015-1035.
6. Kris-Etherton, P.M., Krummel, D., Mackey, S., Borchers, J. and Wood., P.D. (1988) J. Am. Diet. Assoc. 88, 1373-1400.
7. Rutihauser, U. and Goridis, C. (1986) Trends Gen. 2, 72-76.
8. Brenner, R.R. (1984) Prog. Lipid. Res. 23, 69-96.
9. Kreutzberg, G.W., Heymann, D. and Reddington, M. (1986) Cellular Biology of ctoenzymes (Ed.G.W.Kreutzberg),141-164.
10. Saski, T.A., Abe, A. and Sakagami, T. (1983) J. Biol. Chem. 258, 6947-6951.
11. Arch, J.R.S and Newsholme, E.A. (1978) Essays Biochem. 14, 82-123.
12. Guarrnieri, M. and Johnson, R.M. (1970) J. Lipid. Res. 8, 115-123.
13. Clandinin, M.T. (1978) J. Nutr. 108, 273-281.
14. Abuireileh, N.M. and Elson, C.E. (1980) Lipids 15, 918-924.
15. Innis, S.M. and Clandinin, M.T. (1981) Biochem. J. 193, 155-167.
16. McMurchie,E.J. and Raison, J.K. (1979) Biochim. Biophys. Acta. 554, 364-374.
17. Bourre, J.M., François, M., Youyou, A., Dumont, O., Picciotti, M., Pascal, G and Durand G. (1989) J. Nutr. 119, 1880-1892.
18. Youyou, A., Durand, G., Pascal, G., Piciotti, M., Dumont, O. and Bourre, J.M. (1986) J. Neurochem. 46, 224-228.
19. Bourre, J.M., Bonneil, O., Piciotti, M., Nalbonne, G. and Lafont, H. (1988) Biochim. Biophys. Acta. 960, 458-461.
20. Bourre, J.M., Chanez, C., Dumont, O. and Flexor, M.A. (1982) J. Neurochem. 38, 643-649.
21. Chandrasekhara, N. and Ananth Narayan, K. (1970) J. Nutr. 100, 447-480.
22. Awad, A.B. and Chattopadhyay, J.P. (1983) Mechanisms of aging Development. 22, 1151-1158.
23. Flier, J., Lokesh, B.R. and Kinsella, J.E. (1985) Nutr. Res. 5, 277-283.
24. Lindberg, O. and Ernster, L. (1956) Method of Biochemical Analysis (Glick. D ed.) 7-9.
25. Cammer, W., Sirota, S.R., Zimmerman, T.R Jr. and Norton, W.T. (1980) J. Neurochem. 35, 367-376.
26. Lowry, O.H., Rosebrough, N.J., Fan, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
27. Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497-509.
28. Lepage, G. and Roy, C.C (1986) J. Lipid. Res. 27, 114-120.
29. Holman, R.T. (1971) Prog. Chem. Fats Other Lipids IX. 115-118.
30. Crawford, M.A., Casperd, N.M. and Sinclair, A.J. (1976) Comp. Biochem. Physiol. 54B, 395-401.

31. Roland, D.A., Sr. and Edwards, Jr. H.M. (1971) J. Nutr. 101, 1683-1694
32. Chandrasekhara, N. and Ananth Narayan, K. (1970) J. Nutr. 100, 477-480.
33. Brivio-Haugland, R.P., Loius, S.L., Musch, K., Waldeck, N., and Williams, M.A. (1976) Biochim. Biophys. Acta. 433, 150-163.
34. Bernsohn, H. and Spitz, F.J. (1974) Biochem. Biophys. Res. Commun. 57, 293-298.
35. Bourre, J.M., Homayoun, P., François, M., Clément, M., Dumont, O., Piccioti, M., Pascal, G. and Durand, G. (1987) In Phospholipids in the Nervous System: Biochemical and Molecular Pathology. Fidia Research Series, Frontiers in Neuroscience, p. 55.
36. Dipple, I. and Houslay, M.D. (1978) Biochem. J. 174, 179-190.
37. Awad, A.B. and Chattopadhyay, J.P (1983) 113, 1878-1884.
38. Loius, S.L., Brivio-Haugland, R.P. and Williams, M.A. (1976) J. Supr. Molecular. Str. 4, 487-796.
39. Johannsson, A., Smith, G.A . and Metcalfe, J.C. (1981) Biochim. Biophys. Acta. 641, 416-421.
40. Lowenstein, J.M., Yu, M.K., Naito, Y. (1983) "In Regulatory Function of Adenosine". Proceedings of the International Symposium of Adenosine. Charlottesville, VA. (Berne, R.M., Rall, R.T. and Rubio, R., eds) 117-131, Martiness Nijhoff Publishers, Boston.
41. Yannarell, A. and Aronson, N.N. (1973) Biochim. Biophys. Acta. 311, 191-204.
42. Phillis, J.W. and Wu, P.H. (1983) In Regulatory Function of Adenosine (Berne, R.M., Rall, T.W. and Rubio, R. eds) 419-437, Martinus Nijhoff Publishers Boston, MA.
43. Sun, A.S. and Holland, J.F (1985) Basic Life Sci. 35, 163-180
44. Ross, R. (1986) N. Eng. J. Med. 314, 488-500.