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DIETARY LIPIDS AND 5'-NUCLEOTIDASE ACTIVITY OF RAT CELL PLASMA MEMBRANES

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SUMMARY: Three groups of adult rats were fed black currant or olive oils or a 1:1 mixture of the two for three months. Feeding black currant oil diets, high in 18:2 (n-6), 18:3 (n-6), 18:3 (n-3), increased the heart and liver plasma membrane content of linoleic and arachidonic acid with a concomitant decrease of oleic acid. PUFA, n-3 and n-6 content and the bilayer lipid fluidity as examined by measuring the fluorescence anisotropy of diphenylhexatriene were not significantly affected. On the contrary, the 5'-nucleotidase of liver membrane of rats fed black currant diets was lower than that observed in membranes of liver from olive oil fed rats. Therefore it is concluded that PUFA and n-3/n-6 ratio as well as membrane fluidity do not influence the 5'-nucleotidase activity. It is suggested that the activity is sensitive to the amount of a specific fatty acid of the membrane (i.e., oleic or arachidonic acid) and/or to lipid supplementation which can influence the eicosanoids metabolism.

5'-Nucleotidase (E.C. 3.1.3.5, 5'-ribonucleotide phosphorylase) is a widely distributed enzyme which catalyses the hydrolysis of nucleoside 5'-monophosphates into nucleosides and phosphate. In vertebrate tissues four types of 5'-nucleotidase have been detected, three of which being found in the cytoplasm and one being membrane-bound and highly concentrated in the plasma membranes of cells (1,2). The membrane-bound isoform is thought to be an ectoenzyme and the major contributor to the cascade that completely hydrolyses extracellular ATP to adenosine (3). It occurs as a surface located protein and is anchored to the membrane via glycosyl phosphatidylinositol at its C-terminus (4). Additionally, membrane anchoring of the enzyme via transmembrane segments has also been suggested (5-7). Studies on the physical properties of 5'-nucleotidase with special emphasis on its lipid environment seem to be ambiguous. In several reports, a modulation of the enzyme activity either by phospholipids (8-10), cholesterol (11,12) or drugs affecting

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membrane fluidity (13) has been described; in others, no effect of the phospholipid or acyl group composition or drugs on the activity or kinetic properties of 5'-nucleotidase has been observed (14-17).

Recently, investigating the effects of dietary lipids on microsome fatty acid composition and functions, we found that the activity of 5'-nucleotidase was significantly different in organs of animals treated with diets differing in fatty acid composition (18). The diets used in these experiments differed mainly since one diet contained only traces of (n-3) fatty acids (olive oil supplementation), the other was high in (n-3) fatty acids (fish oil supplementation). In order to better define the relationship between dietary oils, physical properties and 5'-nucleotidase activity of membranes, we investigated and we report in this communication the effects observed by feeding rats with graded amounts of olive and black currant oil, which mainly differ in PUFA, oleic and linoleic acid content.

MATERIALS AND METHODS

All chemicals used were purchased from Sigma Chemical Co. Ltd., St Louis, MO, USA and all solvents, of analytical grade, were purchased from Carlo Erba, Milano, Italy.

Three groups of 8 male Wistar rats weighting initially 500 g were obtained from C. River, Italy. The rats were fed ad libitum for three months on a conventional lipid-free diet having the following composition per 100 g: casein, 30g, sucrose and starch, 64g, salt mixture, 4g, cellulose, 2g, choline chlorohydrate and vitamins, 0.1g. Each basic experimental diet was supplemented with 10% by weight of either olive, black currant or a 1:1 (w/w) mixture of the two above oils. The fatty acid composition of the dietary fats are shown in Table 1.

Plasma membrane-enriched fractions were prepared according to (19) from hearts and livers rapidly removed from sacrificed rats. All operations were carried out at 4°C.

Protein concentration of the plasma membrane samples was determined by the biuret method (20) in the presence of 1% deoxycholate; bovine serum albumin was used as a standard.

TABLE 1

Fatty acid composition of oils (mol %)

fatty acid	olive oil	olive/ black currant oil	black currant oil
16:0	13.9	17.8	9.3
16:1	1.3	0.9	0.8
18:0	3.7	2.7	2.1
18:1 (n-9)	72.8	44.8	16.6
18:2 (n-6)	7.2	24.6	41.8
18:3 (n-6)	-	7.3	13.9
18:3 (n-3)	1.1	6.6	12.9
18:4 (n-3)	-	1.3	2.6
PUFA	8.3	39.8	71.2
n-6 / n-3	6.5	4.0	3.6
PUFA/SAT ratio[a]	0.5	1.9	6.2

[a]Polyunsaturated / saturated fatty acid ratio.

The 5'-nucleotidase activity was assayed spectrophotometrically according to (21) and/or by measuring the phosphate released from AMP (22) as previously described (18).

Plasma membrane lipids were extracted by the method of Folch et al. (23), methylated and analyzed as in (18).

Steady-state fluorescence anisotropy measurements were made using a FP-777 Jasco spectrofluorometer. The fluorescent probe DPH (diphenylhexatriene, Aldrich) at 10^{-6} M (final concentration) was added to the membrane suspension containing 0.05 mg protein per ml of 50 mM Tris-HCl, pH 7.5. Excitation of diphenylhexatriene was at 360 nm and emission was recorded at 430 nm. Ten determinations of each parameter were averaged for each membrane preparation. Fluorescence anisotropy was calculated as in (24).

RESULTS AND DISCUSSION

Administration of lipids to the rats as in our experimental conditions had no effects either on health or on body and organ weights. The fatty acid pattern of plasma membrane enriched fractions from heart and liver were affected to a low extent; no significant differences among the various dietary groups in most of the fatty acid content was measured (Table 2). However the oleic acid content of liver plasma membranes from black currant fed rats was half that from olive oil fed rats, reflecting the content of 18:1 in the

TABLE 2

Fatty acid composition (mol %) of the liver from animals fed the three different oil diets: comparison with heart

fatty acid	liver			heart		
	olive	olive/ black currant	black currant	olive	olive/ black currant	black currant
16:0	22.2	24.9	24.0	17.9	17.0	17.6
16:1 (n-9)	2.5	2.3	3.2	1.4	1.2	1.9
18:0 (n-9)	14.5	14.4	13.2	18.9	18.0	18.4
18:1	20.0	15.4*	10.5***	14.5	14.4	10.6*
18:2 (n-6)	14.1	12.3	13.6	9.6	14.3***	14.8*
20:3 (n-6)	0.3	0.6	1.0***	0.2	0.4**	0.4**
20:4 (n-6)	14.1	17.3*	18.2*	17.7	17.3	16.7
20:5 (n-3)	0.7	0.4	1.0*	0.2	0.6**	0.5**
22:4 (n-6)	2.3	2.4	1.9	2.8	2.4	2.5
22:5 (n-6)	1.0	0.4	1.0	1.0	0.4*	0.6
22:5 (n-3)	-	0.4***	0.9***	-	1.0***	1.1***
22:6 (n-3)	6.8	5.5	6.2	11.0	9.4	8.2**
Sat FA[1]	36.7	39.3	37.2	36.8	35.0	36.0
mono FA[2]	22.5	17.7*	13.7***	15.9	15.6	12.5
PUFA[3]	39.3	39.3	37.8	42.5	45.8	44.8
UI[4]	1.7	1.6	1.8	1.9	1.9	1.8
n-3	7.5	6.3	8.1	11.2	11.0	9.8
n-6	31.8	33.0	35.7	31.3	34.8	35.0
n-6/n-3	4.4	5.2	4.3	2.8	3.2	3.6

Each value is the mean of two determinations on eight different organs.
Symbols of significance (* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$) are expressed in comparison with olive oil in each tissue.
[1]Saturated fatty acids ; [2]Monounsaturated fatty acids; [3]Polyunsaturated fatty acids; [4]Unsaturation index is the sum of the mole fraction of each fatty acid times the number of double bonds of the molecule.

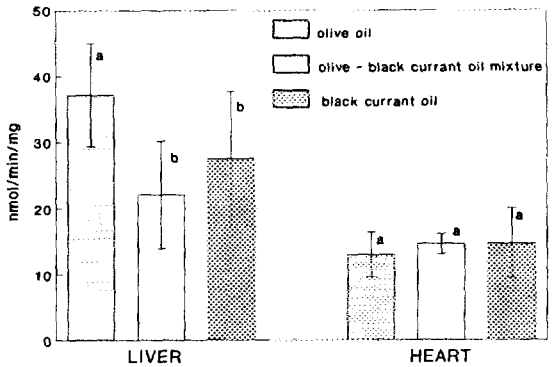

Fig.1. 5'-nucleotidase activity of plasma membranes isolated from rats fed diets containing olive oil, black currant oil or a 1:1 mixture of the two.
Values are means ± SD, n=8. Within the same organ, means not sharing a common letter are significantly different at $p < 0.05$ or less.

diets (Table 1). In a lower extent this occurs also in the plasma membrane from heart cells. The content of 18:2(n-6) of diet was not reflected in the fatty acid pattern of liver plasma membrane, and it was only in part reflected at the heart membrane level.

Significant changes in the liver membrane content of 20:3(n-6) and in 20:4(n-6) was also observed. In particular the arachidonic acid content in liver membranes from black currant fed rats was nearly 30% higher than that found in rats fed olive oil, whereas an intermediate value was observed in rats fed the olive oil-black currant mixture. No significant difference was observed in the arachidonic acid content of heart plasma membranes from rats fed the different diets. More detailed analysis of the fatty acyl pattern of membranes from various tissues will be presented elsewhere (manuscript in preparation).

In addition to modifying membrane fatty acid composition, dietary lipid also significantly altered the 5'-nucleotidase activity of plasma membrane liver cells (Fig.1). The specific activity of the enzyme from olive oil fed rats was significantly higher than in the membranes from animals consuming black currant or black currant/olive oil diets (Fig.1). Under our experimental conditions both the unsaturation index of liver membrane fatty acids and the saturated fatty acid content fluctuated within a narrow range of values (1.7-1.8 and 36.7-39.3 respectively): nevertheless, since a dependence of 5'-nucleotidase from membrane fluidity was reported (10,13), this was investigated by measuring the fluorescence anisotropy of DPH incorporated into the plasma membranes from treated rats. The probe' s steady-state fluorescence anisotropy, which is taken as an index of membrane fluidity (25), is reported in Table 3. Values were virtually identical in all of the olive and black currant oil derived samples, suggesting that, metabolic compensation may have occurred such as to keep constant the plasma membrane fluidity of rats fed the different diets.

TABLE 3

Fluorescence anisotropy of 1,6-diphenyl-1,3,5,-hexatriene (DPH) incorporated into liver plasma membranes

Diet	Anisotropy (r)*
olive oil	0.23 ± 0.03
olive - black currant oil mixture	0.23 ± 0.02
black currant oil	0.22 ± 0.03

*Steady-state fluorescence anisotropy.
Samples were prepared at 25 °C incubating for 45 min 50 µg protein in 1 ml 50mM Tris/Cl, pH 7.5, containing 1 nmol DPH.

A possible relationship between 5'-nucleotidase activity and the n-3 PUFA content of membranes has been suggested (26,27). In our hand this correlation could not be observed since the highest enzyme activity found in liver membranes of olive oil treated rats, occurs when the n-3 content of the membrane has an intermediate (and similar to the others) value. All of the above reasons suggest to analyze whether a direct effect of one (or more) specific fatty acid on the enzyme occurs. A true correlation between the 5'-nucleotidase activity and the fatty acid content of the two membrane examined cannot be found, however the enzyme activity has the maximum value in liver of olive oil fed rats where the oleic acid content is maximal and the arachidonic acid content is minimal (Table 2 and Fig. 1).

In conclusion our results demonstrate that dietary lipids can influence the activity of 5'-nucleotidase in the liver. This effect can not be related to the physical state of the membrane (fluidity), nor to the composition in terms of unsaturation index, nor to (n-3), (n-6) or PUFA content of the membrane, but to an oleic acid rich diet (which is followed by a relatively high content of monounsaturated fatty acids in the membrane) and/or to a linoleic acid poor diet (resulting in a relatively low content of arachidonic acid in the membrane). The implications of changed 5'-nucleotidase activity following particular lipid dietary uptake might be of potential significance. In fact both the nucleotides supplying the substrate to the membrane-bound 5'-nucleotidase and its major product (adenosine) are molecules capable of inducing a variety of physiological functions including vasodilation, inhibition of the immune and inflammatory response (28-30). Furthermore, the possibility that 5'-nucleotidase might be involved in triggering cells growth and differentiation has been suggested (31) and this may have implications in atherogenesis (32). Most of these processes are also influenced by vasoconstrictors, prothrombotic and proinflammatory eicosanoids (33,34) which are sinthesized endogenously from linoleic acid via arachidonic acid (35). Then it is likely that the activity of 5'-nucleotidase and that of enzymes

catalyzing reactions involved in eicosanoids synthesis are controlled under the different dietary regimen, such as to supply the correct balance of extracellular nucleotides, adenosine and eicosanoids.

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