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Eicosapentaenoic and Docosahexaenoic Acids Reduce Arachidonic Acid Release by Rat Kidney Microsomes

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The effects of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) on the phospholipase A_2 (PLA_2)-mediated release of arachidonic acid (AA, 20:4n-6) were studied in kidney microsomes from rats fed diets containing sunflower oil (SO) or fish oil (FO) concentrate for 11 months. The amounts of AA released by the endogenous PLA_2 enzyme were significantly lower by 38% in the FO, compared to the SO-fed rats (23.2 nmol versus 60.7 nmol AA released/mg protein/h in the FO- and SO-treated groups, respectively). The FO-derived microsomes released less linoleic acid (LA, 18:2n-6) and adrenic acid (22:4n-6), but larger amounts of the n-3 fatty acids, including EPA, DHA, docosapentaenoic acid (DPA, 22:5n-3), and 20:4n-3 than the SO-derived microsomes. A similar replacement of the AA and adrenic acid with the n-3 fatty acids including EPA and DHA was also observed in the microsomal phospholipid fraction from the FO-fed rats relative to the SO-treated group. The results suggest that the PLA_2-mediated release of AA is reduced and that of EPA is increased in compensation for AA decline in kidney microsomes from FO-fed rats (0.7 nmol EPA/mg protein/h versus 22.7 nmol EPA/mg protein/h for the SO and FO-treated groups). Replacement of the n-6 with n-3 fatty acids may explain the reduced synthesis of the AA-derived prostaglandins and the concomitant rise in the EPA-derived prostaglandins observed in kidneys of FO-treated rats.

Keywords: Arachidonic acid, Dietary eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), Phospholipase A_2, Rat kidney microsomes.

Introduction

The phospholipase A_2 (PLA_2) enzyme has been reported to hydrolyze fatty acids from the sn-2 position of phospholipids, thereby releasing equimolar amounts of predominantly free fatty acids and lysophospholipids containing mainly saturated fatty acids in the sn-1 position (Chang *et al.*, 1987). PLA_2 activity is reportedly stimulated in MDCK, glomerular mesangial, and inner medullary collecting duct cells by factors including angiotensin II (Ag II), anoxia, arginine vasopressin (aVp), bradykinin, epidermal growth factor (EGF), interleukin 1 (IL-1), and platelet activating factor (PAF); this activation may depend on a prior activation of phospholipase C or may proceed via a direct interaction between the PLA_2 enzyme and a G protein-regulated receptor (Bonventre *et al.*, 1990; Cantiello *et al.*, 1990; Gronich *et al.*, 1990; Teitelbaum, 1990; Nakamura *et al.*, 1991; Portilla *et al.*, 1992). Decreased PLA_2 activity has been reported in kidney medullary slices incubated in hyperosmolar salt and urea-containing solutions (Craven and DeRubertis, 1983).

Results from numerous studies have indicated that in the kidney the PLA_2 enzyme is important in the regulation of eicosanoid production. These studies have shown that increased dietary intakes of eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids, present in enriched amounts in fish oils and their concentrates, are associated with a reduced synthesis of the series 2, or arachidonic acid (AA)-derived, eicosanoids and concomitantly increased synthesis of the series 3 or EPA-derived, in this tissue (Schoene *et al.*, 1981; Croft *et al.*, 1985; Barcelli *et al.*, 1988; Abeywardena *et al.*, 1991; Maranesi *et al.*, 1991). It is of interest to note that both EPA and DHA have been reported to inhibit AA uptake and subsequent incorporation into phospholipids from human platelets and monocytes (Fischer and Weber, 1983; Morita *et al.*, 1983; Weaver and Holub, 1985). Since eicosanoid synthesis is regulated in part by the availability of free AA

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in kidney membranes, the present work was conducted to evaluate the influence of the n-3 fatty acids (notably EPA and DHA) on the PLA_2-mediated release of AA, EPA, and DHA in rat renal microsomes.

Materials and Methods

Animals and diets Male weanling rats (Charles River, St. Constant, Quebec, Canada) having an average weight of 60 g were fed (*ad libitum*) semipurified diets containing sunflower oil (SO) rich in linoleic acid (LA, 18:2n-6) or MaxEPA, a fish oil concentrate (FO) enriched with EPA plus DHA (R.P. Scherer, Windsor, Ontario, Canada) and supplemented with sufficient SO to satisfy the nutritional requirements for LA in the rat. Details of these diets have been previously reported (Yeo *et al.*, 1989). The rats were housed individually in stainless steel cages in a temperature- and humidity-controlled room with a 12 h light–dark photoperiod and were weighed once weekly for 11 months.

Preparation of kidney microsomes The kidneys from rats sacrificed by cervical dislocation were quickly removed, decapsulated, weighed, and individually homogenized in 4 vol of an ice-cold 0.25 M sucrose, 1 mM EDTA, and 0.1 M Tris·HCl buffer (pH 7.4) using a Potter-Elvehjem homogenizer. Microsomal fractions from these kidney homogenates were obtained by differential centrifugation using modified procedures described for rat liver microsomes (Holub, 1977) and the resulting pellet was resuspended in a 0.1 M Tris·HCl buffer (pH 8.0) containing 10 mM dithiothreitol, 0.5 mM EGTA, and 10 mM $MgCl_2$ (Woodard *et al.*, 1987). Protein contents of the microsomes were detemined by the colorimetric procedure of Lowry (1951) using bovine serum albumin as the standard.

PLA_2 assay procedure The standard assay procedure used to monitor the release of AA and other fatty acids by the PLA_2 enzyme consisted of a 0.1 M Tris·HCl buffer, pH 8.0, containing 80 μM of the lipoxygenase-cyclooxygenase inhibitor BW44C (3-amino-1-[M-trifluoromethylphenyl]-2-pyrazoline, Wellcome Research Laboratories, Breckenham, UK) (Smith *et al.*, 1985), 4 mM $CaCl_2$, and 1000 μM of the reconstituted enzyme protein in a final volume of 0.5 ml. The microsomes containing the endogenous PLA_2 enzyme were incubated at 37°C in a shaking water bath for 1 h after which the reaction was stopped by the addition of 4 ml of chloroform:methanol (2:1, v/v) (Kawaguchi and Yasuda, 1986). Control release of fatty acids was assessed by incubating the reconstituted microsomal protein pellet with a specific phospholipase A_2 inhibitor, 500 μM quinacrine dihydrochloride (6-chloro-9-[4-diethyl-amino]-1-methylbutyl amino-2-methoxyacridine dihydrochloride (Sigma Chemical Co., St. Louis, USA) in the presence and absence of calcium (Jain and Jahagirdar, 1985; Loffler *et al.*, 1985).

Thin-layer chromatography of the non-esterified fatty acids and total phospholipid fractions and gas liquid chromatographic analyses of the fatty acid methyl esters Five micrograms of pentadecanoic acid (C15:0, NuChek Prep, Elysian, USA) were added to each incubation tube as an internal standard and the lipids extracted by the Folch procedure (Folch *et al.*, 1957). The neutral lipids, including the phospholipid and free fatty acid fractions, were separated by thin-layer chromatography on pre-coated silica gel 60 plates (E. Merck, Darmstadt, Germany), and developed in a heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) solvent system. The bands were scraped from the plates after spraying with 2,7-dichlorofluorescein and visualization under UV light, and heated in 6% methanolic H_2SO_4 (v/v) at 80°C for 1.5 h. Following extraction with pet ether, the fatty acid methyl esters (FAME) were separated by gas chromatography using a Hewlett Packard model 5890A gas chromatograph, equipped with a DB225 megabore column (Chromatographic Specialties, Brockville, USA). The operating conditions for these isothermal runs have been previously described (Yeo *et al.*, 1989). The FAME were identified by comparison of retention times with known standards.

Peak areas and weight percentage of the fatty acids were computed by a Spectra-Physics 4100 SP integrator. Results are expressed as mean ± S.E.; the Student's t-test was used to assess the level of significance of the mean differences between the SO and FO groups.

Results

Food intakes, final body weights, and kidney weights were similar in the SO- and FO-fed groups; gross anatomical examination of the kidneys at sacrifice revealed no evidence of abnormal pathology.

An average of 35.5 ± 5.1 nmol AA/mg protein/h was released following incubation of the kidney microsomes from the SO group; significantly lower levels of AA (9.8 ± 2.6 nmol) were generated during incubation of microsomes from the FO group (Fig. 1). Release of free AA from the SO and FO groups fell to 10.4 and 4.3 nmol AA, respectively, when the incubation media did not contain added calcium, and to 5.6 and 4.6 nmol AA released in the presence of 500 μM quinacrine dihydrochloride. These decreases in enzyme potential are consistent with the reported calcium dependency of the PLA_2 enzyme and its inhibition by quinacrine (Gronich *et al.*, 1990; Teitelbaum, 1990; Nakamura *et al.*, 1991; Smith *et al.*, 1985; Kawaguchi and Yasuda, 1986; Jain and Jahagirdar, 1985; Loffler *et al.*, 1985). The net PLA_2-mediated release of free AA was determined to be 60.7 ± 9.9 and 23.2 ± 3.4 nmol/mg protein/h for the SO and FO group, respectively, under the conditions employed.

Compositional profiles of the free fatty acids released by the PLA_2-mediated enzyme from kidney microsomes of the SO- and FO-fed groups are presented in Table 1. These data show that microsomes from the SO animals released a total of 113.3 ± 21.9 nmol n-6 fatty acids, of which AA was the principle fatty acid followed by somewhat smaller amounts of LA and very minor amounts of adrenic acid (22:4n-6). Small amounts of the n-3 fatty acids were released from the SO-treated microsomes (8.0 ± 1.8 nmol). Of these, DHA was the major n-3 fatty acid released, followed by 18:3 and 18:4 (data not shown) as well as 20:4 and EPA.

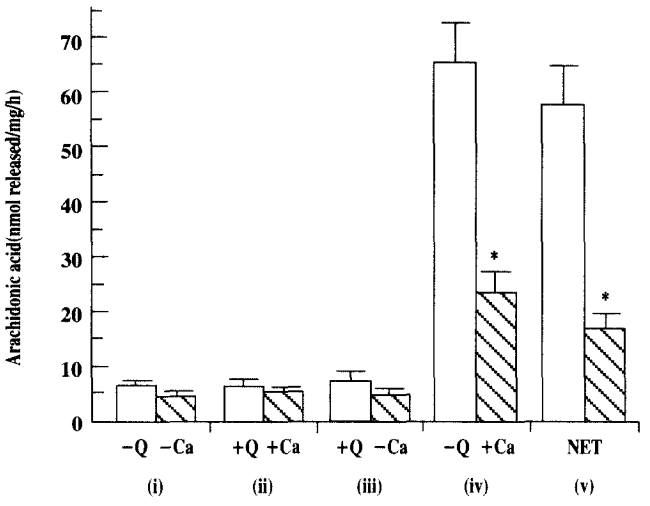

Fig. 1. Arachidonic acid (AA) released by kidney microsomes from rats fed sunflower oil (SO)- or MaxEPA oil (FO)-based diets. The several combinations of quinacrine (Q) and calcium present in the incubation medium are depicted along the abscissa as: −Q−Ca (i), +Q+Ca (ii), +Q−Ca (iii), and −Q+Ca (iv). The net AA release [NET, (v)] was obtained by subtracting the amount of AA released by the calcium-dependent phospholipase A_2 (ii) from the amounts released by the calcium-dependent enzyme not inhibited by quinacrine (iv). Clear bars represent AA release by the kidney microsomes from the SO group; release of AA by the microsomes from the FO rats is indicated by the striped bars. Results are means ± S.E. for 11–12 kidneys from each dietary treatment. *Significantly different from the SO-derived microsomes, $P<0.05$.

The total amount of the n-6 fatty acids released by microsomes from the FO-fed rats was 36.4 ± 4.4 nmol fatty acid, or 32% of that observed in the SO-treated group; the amounts of AA, LA, and adrenic acid released from the FO-derived microsomes were 38, 24, and 5%, respectively, of the levels released by the SO-fed rats. Amounts of 20:4n-3, EPA, 22:5n-3 (docosapentaenoic acid; DPA), and DHA released from the FO-treated microsomes were significantly higher than those amounts released by the SO group. The levels of 18:1, 20:1, 22:1, and 24:1 fatty acids tended to be lower in the FO group compared to the SO group, although these differences were not significant (data not shown).

Whereas no differences were found in the amount of total phospholipid between the two dietary groups (data not shown), marked differences in the fatty acid profiles were apparent (Table 2). Microsomes from the FO group showed markedly lower amounts of the n-6 fatty acids, including AA and adrenic acid, in the phospholipid fraction and moderately lower amounts of LA. These changes in the phospholipid fraction were accompanied by concomitant increases in the 20:4n-3, EPA, DPA, and DHA fatty acids.

Table 1. Phospholipase A_2-mediated release of various fatty acids in kidney microsomes of sunflower seed Oil (SO)- and Fish Oil (FO)-fed rats[a]

Fatty acid[b]	Dietary group	
	SO	FO
	nmol fatty acid released/mg protein/h	
Total n-6 series	113.3 ± 21.9	36.4 ± 4.4[c]
18:2 (LA)	46.2 ± 11.3	11.4 ± 1.5[c]
20:4 (AA)	60.7 ± 9.9	23.2 ± 3.4[c]
22:4 (adrenic acid)	2.0 ± 0.4	0.1 ± 0.1[d]
Total n-3 series	8.0 ± 1.8	38.4 ± 5.0[d]
20:4	1.2 ± 0.9[e]	0.4 ± 0.5
20:5 (EPA)	0.7 ± 0.4	22.7 ± 2.8[d]
22:5 (DPA)	0.2 ± 0.1	1.6 ± 0.3[d]
22:6 (DHA)	4.3 ± 0.9	11.9 ± 1.7[d]
n-6/n-3 ratios	24.7 ± 8.6	1.0 ± 0.1[e]

[a] Values given are means ± S.E. for 11–12 kidneys/treatment.
[b] Fatty acids excluded from the table include: 16:0 and 16:1 isomers, 18:0 and 18:1 isomers, 18:3n-3, 18:4n-3, 20:0, and 20:1 isomers, 20:2n-6, 20:3n-6, 20:3n-3, 22:0, and 22:1 isomers, 24:0 and 24:1 isomers.
[c] Significantly different from the SO group, $P<0.01$.
[d] Significantly different from the SO group, $P<0.001$.
[e] Significantly different from the SO group, $P<0.05$.

Table 2. Fatty acid compositions of the total phospholipid in kidney microsomes from Sunflower seed Oil (SO)- and Fish Oil (FO)-fed rats[a]

Fatty acid[b]	Dietary group	
	SO	FO
	nmol fatty acid released/mg protein/h	
Total n-6 series	113.3 ± 21.9	36.4 ± 4.4[c]
Total n-6 series	32.1 ± 2.5	19.0 ± 0.7[c]
18:2 (LA)	5.4 ± 0.5	4.3 ± 0.2[c]
20:4 (AA)	25.6 ± 2.0	14.3 ± 0.6[c]
22:4 (adrenic acid)	0.7 ± 0.1	0.0 ± 0.0[c]
Total n-3 series	1.0 ± 0.3	15.9 ± 0.7[c]
20:4	0.1 ± 0.1	0.6 ± 0.0[c]
20:5 (EPA)	0.1 ± 0.0	10.0 ± 0.4[c]
22:5 (DPA)	0.0 ± 0.0	0.7 ± 0.1[c]
22:6 (DHA)	0.5 ± 0.1	4.7 ± 0.2[c]
n-6/n-3 ratios	43.0 ± 6.2	1.2 ± 0.4[c]
EPA/AA ratio	0.0016 ± 0.0	0.7 ± 0.0[c]
DHA/AA ratio	0.02 ± 0.001	0.3 ± 0.0[c]
EPA/DHA ratio	0.2 ± 0.1	2.1 ± 0.1[c]

[a] Values given are means ± S.E. for 9–12 kidneys/treatment.
[b] Fatty acids excluded from the table include: 16:0 and 16:1 isomers, 18:0 and 18:1 isomers, 18:3n-3, 18:4n-3, 20:0, and 20:1 isomers, 20:2n-6, 20:3n-6, 20:3n-3, 22:0, and 22:1 isomers, 24:0 and 24:1 isomers.
[c] Significantly different from the SO group, $P<0.001$.

Discussion

Results from this study indicate that prolonged ingestion of an EPA plus DHA-enriched diet by rats is associated with a significant reduction in the PLA_2-mediated release of the n-6 fatty acids in kidneys microsomes from FO-fed rats. Also, these microsomes showed decreased AA contents in both the free fatty acid (Fig. 1 and Table 1) and the phospholipids fractions (Table 2). These results are in agreement with the reported n-3 fatty acid replacement of AA in the total phospholipid fractions from kidneys of rodents fed FO-based diets (Huang *et al.*, 1986; Nassar *et al.*, 1986; Swanson *et al.*, 1987; Kim *et al.*, 1997). The attenuation of PLA_2-mediated AA release observed in the present experiment may be related to a decreased availability of AA-containing phospholipids in the FO-derived microsomes of rat kidney, although PLA_2 spreads widely in animal tissues (Baek and Chang, 1992; Park *et al.*, 1997). The results reported herein do not directly examine this hypothesis, but it is of interest that LA release from the FO-derived microsomes was markedly lower (Table 1) than that from the SO group, although the amounts of this fatty acid present in the phospholipid fractions were similar in the SO and FO groups (Table 2). This finding suggests that in the case of LA, and perhaps the other n-6 fatty acids, substrate availability is not the sole factor regulating the PLA_2 enzymatic activity. The results from this and other studies have shown that LA levels in renal phospholipids are relatively unaffected by increased intakes of EPA plus DHA-enriched diets by rodents (Huang *et al.*, 1986; Nassar *et al.*, 1986; Swanson *et al.*, 1987) and it is possible that LA levels are preserved in this tissue by the inhibitory effects of EPA and DHA on the conversion of LA to AA and adrenic acid (Yeo *et al.*, 1989; Choi *et al.*, 1993; Park *et al.*, 1995).

The data presented in Table 1 show that adrenic acid hydrolysis is also reduced in the FO-derived microsomes and this finding suggests that the kidney PLA_2 enzyme can hydrolyze this fatty acid, as well as AA, from the sn-2 position of a phospholipid substrate. The lack of a strict specificity for AA at the sn-2 position has been reported for the kidney PLA_2 enzyme in rat medullary and cortical tissues (Kawaguchi and Yasuda, 1986). Also, the presence of an arachidonyl-specific PLA_2 enzyme has not been consistently demonstrated in this tissue (Gronich *et al.*, 1990; Fujimori *et al.*, 1992).

The results obtained in the present study indicate that PLA_2-mediated AA release is reduced and that of EPA is increased in kidney microsomes from FO-fed rats. As a major precursor of eicosanoid, AA has been regarded as a biologically significant fatty acid incorporated into membrane phospholipids, and increased AA-derived eicosanoid productions have been observed following PLA_2 stimulation in a variety of kidney cells (Coyne *et al.*, 1989; Pfeilschifter *et al.*, 1989), whereas ingestion of EPA plus DHA-enriched diets has been associated with an attenuated production of these eicosanoids in normal and aging rats (Schoene *et al.*, 1981; Croft *et al.*, 1985; Barcelli *et al.*, 1988; Abeywardena *et al.*, 1991; Maranesi *et al.*, 1991) and in several animal models of kidney disease (Kelley *et al.*, 1985; Scharschmidt *et al.*, 1987; Watson *et al.*, 1988; Clark *et al.*, 1990; Scharschmidt *et al.*, 1990; Thaiss *et al.*, 1990; Wheeler *et al.*, 1991; Logan *et al.*, 1992). Recent studies showing that EPA and DHA can directly inhibit the PLA_2-regulated sodium channel activity in polarized renal epithelial cells (Cantiello *et al.*, 1990) underline the importance of these interactions among the PLA_2 activity, the phospholipid n-3 fatty acid composition, and the biological function of this tissue.

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