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Astrocytes in Culture Require Docosahexaenoic Acid To Restore the n-3/n-6 Polyunsaturated Fatty Acid Balance in Their Membrane Phospholipids

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Docosahexaenoic acid (DHA), the main n-3 polyunsaturated fatty acid (PUFA) in membranes, is particularly abundant in brain cells. Decreased cerebral concentrations of DHA, resulting from dietary n-3 deficiency, are associated with impaired cognitive function. Because the cellular causes of this impairment are still unknown, we need in vitro models that mimic the variations in n-3/n-6 PUFA seen in vivo. We have compared the PUFA profiles of hamster astrocytes cultured in medium supplemented with long-chain PUFA [DHA and/or arachidonic acid (AA)] with those of brain tissue from hamsters fed an n-6/n-3 PUFA-balanced diet or one lacking n-3 PUFA. Astrocytes were obtained from the brain cortex of newborn hamsters and cultured in minimum essential medium + 5% fetal calf serum (FCS) supplemented with DHA and/or AA for 10 days. The astrocytes cultured in medium + FCS had low n-3 PUFA contents, comparable to those of brain tissue from hamsters fed an n-3-deficient diet. We have shown that astrocytes grown in medium supplemented with DHA and/or AA, plus α-tocopherol to prevent lipid peroxidation, incorporated large amounts of these long-chain PUFA, so that the n-6/n-3 PUFA compositions of the phosphatidylethanolamine and phosphatidylcholine, the two main classes of membrane phospholipids, were greatly altered. Astrocytes cultured in medium plus DHA had a more physiological n-3 status, grew better, and retained their astrocyte phenotype. Thus astrocytes in culture are likely to be physiologically relevant only when provided with adequate DHA. This reliable method of altering membrane phospholipid composition promises to be useful for studying the influence of n-6/n-3 imbalance on astrocyte function.

Key words: astrocytes; membrane phospholipids; docosahexaenoic acid; arachidonic acid; cell culture

Long-chain polyunsaturated fatty acids (LC-PUFA), mainly docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (AA; 20:4n-6), are fundamental constituents of cell membrane phospholipids. Brain cells, including neurons and astrocytes, are specifically enriched in DHA (Bourre et al., 1984, 1992). Both the n-3 and the n-6 series of LC-PUFA are synthesized by the enzymes, elongases, and desaturases acting on essential precursors found in vegetable oils, α-linolenic acid (18:3n-3), and linoleic acid (18:2n-6). Because mammals are unable to produce these precursors, a lack of dietary n-3 PUFA can lead to subnormal concentrations of DHA in the brain, and this is associated with cognitive and visual defects in animals (Bourre et al., 1989; Wainwright, 1992; Moriguchi et al., 2000; Carrié et al., 2002) and in humans (Holman et al., 1982; Uauy and Mena, 2001). Therefore, the low n-3/n-6 PUFA ratio in Western diets may well influence several aspects of brain development, function, and aging.

Despite this evidence of the importance of DHA for normal brain function, very little is known about the role of DHA in brain cells and especially in astrocyte function. Astrocytes are essential for neurotransmission and neuroprotection, because they maintain the composition of the extracellular milieu, respond to neuromediators, and supply energetic substrates to neurons. We postulate that astrocytes are target cells for the DHA in brain because astrocyte activity relies greatly on membrane events and lipid signaling. Altering the amount of DHA in the cell membranes can affect the biophysical properties of membrane-bound proteins (receptors, transporters, and adhesion and cytoskeleton proteins) and change the signaling pathway linked to lipid messengers, notably the release of arachidonic acid and production of eicosanoids

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(Galli et al., 1992; Litman and Mitchell, 1996; Yehuda et al., 1998; Salem et al., 2001; Broughton and Wade, 2002). AA and its eicosanoid derivatives affect essential properties of astrocytes, such as volume regulation (Sanchez-Olea et al., 1995), glutamate and glucose uptake (Yu et al., 1986, 1993), and junctional coupling (Giaume et al., 1991; Martinez and Saez, 1999). Increasing n-6 PUFA content in cultured astrocytes has been shown to decrease cAMP formation induced by dopamine and the uptake of adenosine (Murphy, 1995). The function of the physiological high DHA content of astrocytes membranes is much less clear than that of its n-6 competitor AA. Understanding the implication of DHA in brain cellular mechanisms and especially in astrocyte function requires the development of primary cell culture models that reflect physiological variations of DHA in their membrane phospholipids. This study was carried out to define such a model, using cultured astrocytes from the cortex of newborn hamsters. We assessed the physiological relevance of the in vitro model by comparing the lipid composition of the cells with that of the cerebral cortex of hamsters fed an n-3/n-6 PUFA-balanced diet and hamsters fed an n-3 PUFA-deficient diet. The fatty acid composition of cell membranes was examined, focusing on the two main classes of phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC).

Our findings indicate that astrocytes cultured under classical conditions (5% fetal calf serum) have a very low n-3 PUFA content. We examined the ability of DHA, added to the medium, to restore the natural high DHA content of astrocytes in situ. We also supplemented the medium with a combination of DHA and AA to obtain different n-3/n-6 PUFA profiles in the cells. The effect of the LC-PUFA supplementation on cell culture integrity and the need for cosupplementation with an antioxidant (α-tocopherol) were checked by analyzing cellular lipid peroxidation, cell growth, and expression of the astrocytic phenotype.

MATERIALS AND METHODS

Animals and Diet

Male Syrian hamsters (2–3 months old) were housed under an LD 16:8 cycle (16 hr light/8 hr dark) and fed a basal diet containing 7% lipid supplement. The n-3/n-6 PUFA-balanced diet was supplemented with peanut and rapeseed oils (essential fatty acid content: 21% 18:2n-6 and 5% 18:3n-3), whereas the n-3 PUFA-deficient diet contained peanut oil only (essential fatty acid content: 22% 18:2n-6 and <0.1% 18:3n-3). The experimental hamsters had been born to female hamsters fed the same diets for 2 weeks before mating. All experiments were carried out in accordance with French regulations (Nos. 87 848 and 03056) and NIH guidelines (No. 85-23) for the care and use of laboratory animals. The hamsters were decapitated and aliquots of cerebral cortex immediately collected for lipid extraction.

Culture of Astrocytes

Cultures of astrocytes were prepared from 1–3 day-old Syrian hamsters. The meninges were carefully removed, and the cerebral cortex was dissociated mechanically by passage through a syringe. The resulting homogenate was filtered through 70-μm nylon meshes. The resulting cells were grown in Eagle's minimum essential medium (MEM; Invitrogen France) with 10% fetal calf serum (FCS; Cambrex France), supplied with extra substances to make up the final concentration (vitamin × 2, amino acids × 2, 7 mM glucose; Invitrogen France) and supplemented with 90 mg/ml bicarbonate, 100 IU/ml penicillin, 100 μg/ml strepromycin, and 250 pg/ml fungizone (all products from Invitrogen France), in 75 cm^2 tissue culture flasks at 37°C in a humidified atmosphere of 5% CO_2 in air. The culture medium was changed every 48 hr until the cells were confluent; then, cells were plated out for the experiments.

LC-PUFA Supplementation Protocols

Experiments were conduced to determine the effect of the concentration of DHA in the medium on membrane fatty acid composition, the effect of DHA + AA co-supplementation on membrane fatty acid composition, the effect of α-tocopherol co-supplementation on potential DHA toxicity (lipid peroxidation and cell growth), and the effect of DHA supplementation on cell growth and differentiation. DHA and/or AA supplements were added to the medium of secondary cultures plated at 5,000 cells/cm^2 in MEM + 5% FCS in tissue culture plates (TPP; ATGC France). LC-PUFA (DHA and/or AA) and/or α-tocopherol were added to the culture medium 1 day after plating, then every 48 hr, when the medium was replenished. Sodium salts of DHA or AA (NuChek Prep, Elysian, MN) were first dissolved in FCS and diluted in MEM to final concentrations of 15, 30, or 60 μM as indicated in the figures. α-Tocopherol (99% d-α-tocopherol; Acros Organics France) was first dissolved in ethanol and diluted in FCS (final concentration of ethanol in culture medium 1/10,000). The cultures contained >90% astrocytes, as judged by immunohistochemical staining with antiglial fibrillary acidic protein (GFAP) antibodies directly conjugated to CY3 (Sigma-Aldrich France). Cells were harvested after different times in culture for measurement of lipid peroxidation, growth, and GFAP. The lipid composition of the cells that had been cultured for 10 days was analyzed; cells were washed with phosphate-buffered saline (PBS) containing 50 μM fatty acid-free albumin (Sigma Chimie France) to remove any fatty acids absorbed onto the cell surface, scraped off into PBS-albumin, and centrifuged.

The effects of α-tocopherol and the dose-dependent effects of DHA were determined using cells cultured with vehicle alone (ethanol 1/10,000), α-tocopherol (45 or 70 μM), DHA (30 or 60 μM), or DHA (30 or 60 μM) + α-tocopherol (45 or 70 μM). The effects of DHA plus AA supplements were determined on four sets of cells cultured with α-tocopherol alone (70 μM), α-tocopherol (70 μM) + DHA (30 μM), α-tocopherol (70 μM) + AA (30 μM), or α-tocopherol (70 μM) + AA (15 μM) + DHA (15 μM).

Measurement of Thiobarbituric Acid-Reactive Substances

The amount of lipid peroxides in cultured cells was evaluated by measuring the formation of thiobarbituric acid-reactive substances (TBARS; Wallin et al., 1993). Aliquots of sonicated cells (700 μl) were mixed with 50 μl of 50% trichloroacetic acid

and 75 μl of 1.3% thiobarbiturate in 0.3% NaOH. The mixture was shaken, heated to 80°C for 20 min with gentle shaking, cooled, and centrifuged at 6,000 rpm for 5 min at 4°C. The supernatants containing TBARS were separated, and their absorbance at 540 nm was measured. A standard curve was prepared with diethylacetal malondialdehyde (Sigma France). The results are expressed as fmole equivalents malondialdehyde per milligram protein.

Measurement of Cell Growth

The total DNA contents of the cultured cells after different times in culture were analyzed with the bisbenzimide fluorescence assay (Labarca and Paigen, 1980).

GFAP Western Blotting

GFAP, the specific protein of astrocyte intermediate filaments, is an indicator of astrocyte differentiation in cultured brain cells. We analyzed the overall expression of GFAP by Western blotting to evaluate the impact of DHA supplementation on the expression of the astrocyte phenotype in culture. Cell samples were dissolved in sodium dodecyl sulfate (SDS) sample buffer and sonicated. The protein were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% gel, in parallel with a mixture of molecular weight markers (New England Biolabs, Beverly, MA), then transferred to a polyvinyldene fluoride (PVDF) membrane (Immobilon-P; Millipore, Bedford, MA) in transfer buffer, pH 8.3 (25 mM Tris, 192 mM glycine, and 20% v/v ethanol). Immunoblots were blocked by incubation for 6 hr at 4°C in TBS-T (20 mM Tris, pH 7.6, 0.8% NaCl, and 0.1% Tween 20) containing 5% skim milk powder. They were then incubated overnight at 4°C with primary monoclonal anti-GFAP antibodies (diluted 1:500; ICN Biomedicals France) in TBS-T containing 5% skim milk powder. Membranes were washed in TBS-T and then incubated for 2–3 hr at room temperature with secondary antibody (anti-mouse horseradish peroxidase-conjugated IgG, diluted 1:15,000; Jackson, West Grove, PA) in TBS-T. The membranes were washed again and incubated for 5 min with ECL+ reagents (Amersham France) to detect immunoreactive proteins.

Lipid Extraction and Fatty Acid Analysis

Cells or tissue were placed in chloroform/methanol (2/1, v/v) plus 0.005% butyl-hydroxytoluene. Totals lipids were extracted according to Folch et al. (1957). The lipid phase of each sample was dried under nitrogen and dissolved in eluent (isopropanol/chloroform, 1/2 v/v). The classes of cell phospholipids were separated by solid-phase extraction (Pietsch and Lorenz, 1993) on a 500 mg prepacked aminopropyl cartridge (J.T. Baker, Deventer, The Netherlands) as described by Goustard-Langelier et al. (2000). The two major cell phospholipid fractions, PC and PE, were dried under nitrogen and directly transmethylated. Fatty acid methyl esters were produced by reacting PC for 20 min with 10% boron trifluoride in methanol at 90°C (adapted from Morrison and Smith, 1964) and reacting PE for 4 hr with 10% HCl and 4% dimethoxypropane in methanol at 70°C (Berry et al., 1965). The fatty acid methyl esters were assayed on a 9001 gas chromatograph (Chrompack, Middleburg, The Netherlands) equipped with a retention gap and a CP WAX 52 CB bonded fused-silica capillary column (50 m × 0.25 mm). The instrument responses attributable to fatty acid methyl esters were automatically integrated, and their response factors and equivalent chain lengths were compared with those of standard compounds. All compositions are expressed as percentages by weight of total fatty acids.

Statistical Methods

Statistically significant differences between cell groups were evaluated using GraphPad software for one-way ANOVA, followed by a Newman-Keuls test. Differences between animals were evaluated using Student's *t*-test in the same software package. Data are mean ± SEM for one representative experiment among two or three.

RESULTS

In Vivo Modulation of Hamster Brain Fatty Acid Composition by Dietary n-3/n-6 PUFA

This part of the study establishes a physiological reference for evaluating the relevance of manipulating the fatty acid composition of astrocytes in culture. All fatty acids were analyzed; they are presented by category, and only the most representative PUFA (>2%) are shown individually (Fig. 1).

The hamsters deprived of dietary n-3 PUFA had proportions of n-6 and n-3 PUFA in their brain PE and PC significantly different from those of hamsters fed the n-6/n-3 PUFA-balanced diet. The n-3 PUFA in PE was markedly decreased (–55%), as was the n-3 PUFA in PC (–68%), and the n-6 PUFA contents were increased (+36% in PE and +39% in PC). These changes essentially were due to differences in the relative abundance of DHA (22:6n-3) and docosapentaenoic acid (DPA; 22:5n-6). DPA is the n-6 derivative produced from AA by the microsomal/peroxisomal enzymatic pathway of elongation–Δ6-desaturation–β-oxidation (Sprecher et al., 1995). Therefore, DPA is the n-6 metabolic equivalent of DHA, the n-3 PUFA that is formed from eicosapentaenoic acid (EPA; 20:5n-3) by the same enzymatic pathway. The DHA in PE was 55% lower, and the DHA in PC was 70% lower, in n-3-deficient hamsters than in the hamsters fed the n-3/n-6-balanced diet. In compensation, DPA, which was only a minor PUFA in the brain of hamsters fed the n-3/n-6-balanced diet, had increased markedly to concentrations close to those of DHA (11% of total fatty acids in PE and 3.4% of total fatty acids in PC). In contrast, the AA (20:4n-6) concentration was not altered by the diet, in either phospholipid class. The total PUFA contents of the brains from the n-3-deficient animals and from those fed the n-3/n-6-balanced diet were not significantly different. They represented nearly one-half of the esterified fatty acids in PE (47% in average in both groups) and 12% in PC, which has a much lower PUFA content than PE. Similarly, the total monounsaturated (MUFA) and saturated (SFA) fatty acids in the PE and PC from hamsters fed an n-3-deficient diet and those fed an n-3/n-6-balanced diet were the same.

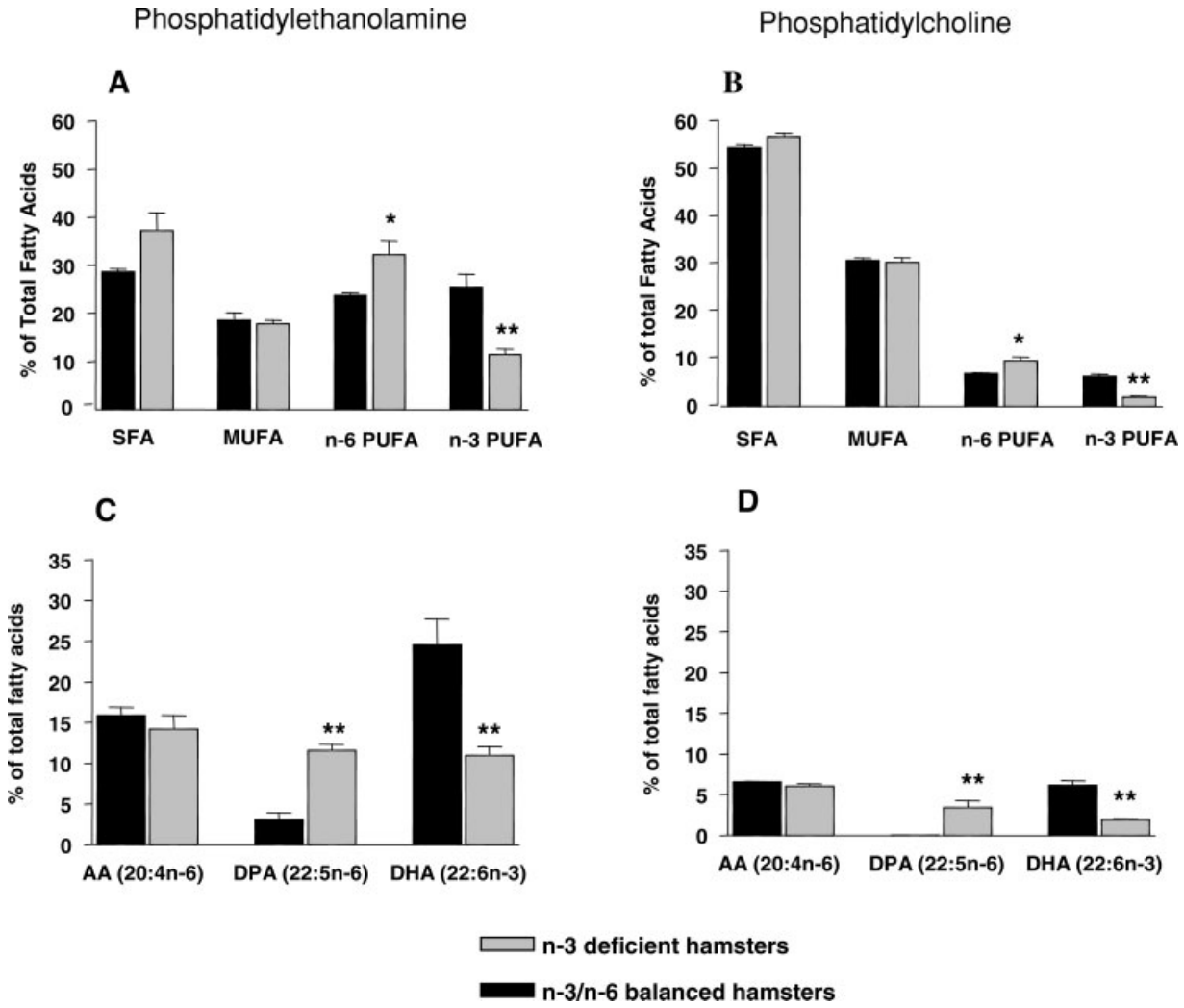

Fig. 1. Effect of dietary n-3 PUFA on the fatty acid compositions of phosphatidylethanolamine (left column) and phosphatidylcholine (right column) of brain tissue from hamster cortex. **A,B:** Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 and n-3 polyunsaturated fatty acids (PUFA). Each bar represents the sum of all detectable fatty acids of the corresponding series. **C,D:** Arachidonic acid (AA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA). The results are in percentage of total fatty acids. $\star P < .05$, $\star\star P < .01$ (Student's *t*-test), significant difference in the concentration of each fatty acid in hamsters fed the n-3/n-6-PUFA balanced diet and in those fed the n-3-deficient diet.

Lack of Effect of α-Tocopherol on Astrocyte Fatty Acid Composition

Because α-tocopherol was added to DHA- and/or AA-supplemented culture medium as an antioxidant, we examined its effect on the fatty acid composition of cultured astrocytes. The fatty acid compositions of cells incubated with α-tocopherol (70 μM) and cells incubated with vehicle (ethanol 1/10,000) were similar (Fig. 2), as were those of cells incubated in a lower concentration of α-tocopherol (45 μM; data not shown). Thus, α-tocopherol per se had no effect on the fatty acid composition of membrane phospholipids, at least at the concentration tested (45 and 70 μM). Cells supplemented with DHA and/or AA were therefore compared with unsupplemented cells in medium containing 70 μM α-tocopherol as controls.

Dose-Dependent Effect of DHA on Astrocyte Fatty Acid Composition (Fig. 2)

Astrocytes grown in DHA-supplemented medium took up DHA and incorporated large amounts of it into PE and PC. The DHA in both phospholipid classes increased significantly with the concentration of DHA in the medium, up to five times the control content in PE and 10 times that in PC, in cells incubated in medium containing the higher concentration of DHA (60 μM). However, doubling the concentration of DHA in the medium (from 30 to 60 μM) caused a 66% increase in DHA in PC but

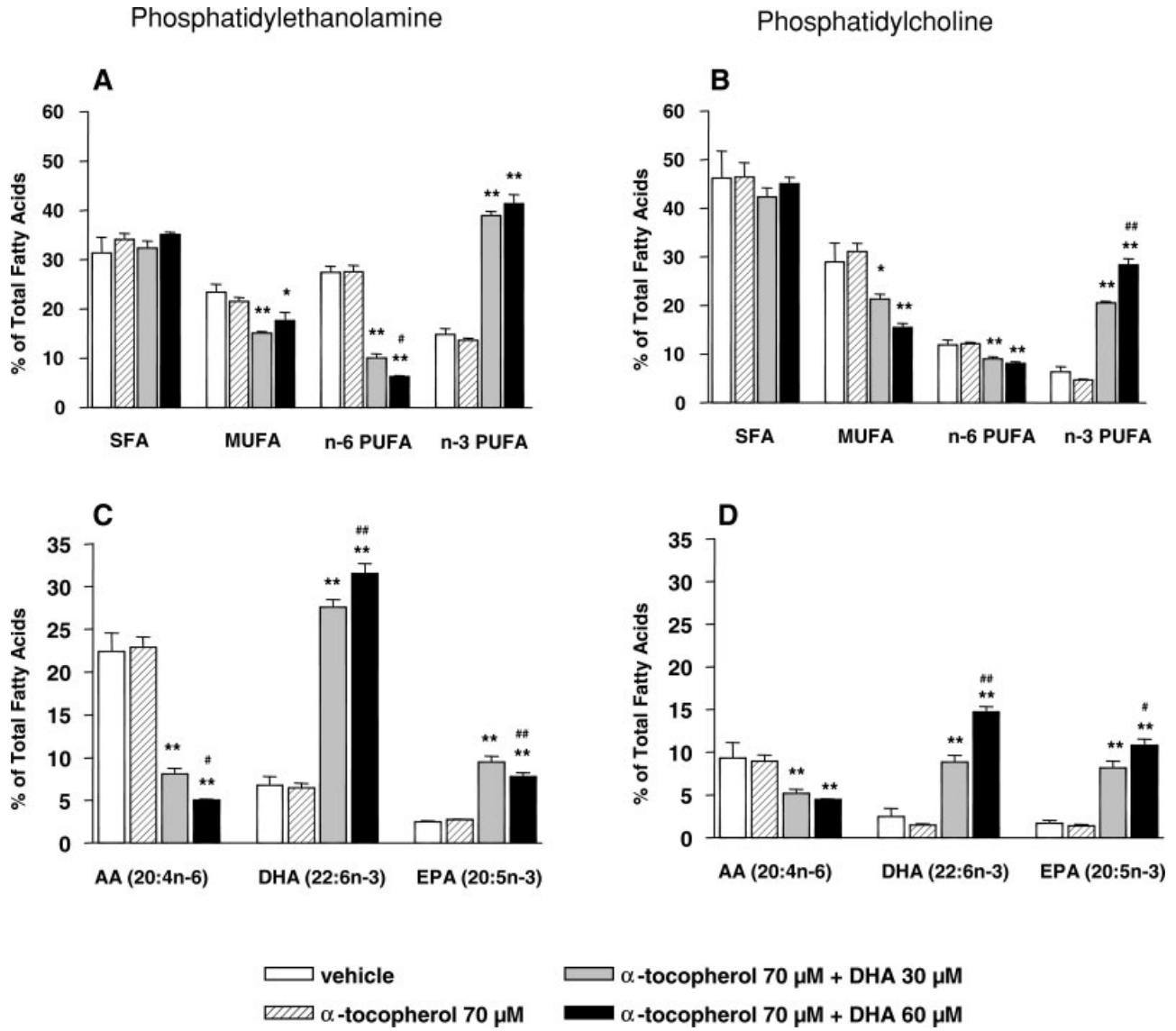

Fig. 2. Fatty acid compositions of phosphatidylethanolamine (left column) and phosphatidylcholine (right column) of cultured astrocytes. **A,B:** Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 and n-3 polyunsaturated fatty acids (PUFA). Each bar represents the sum of all detectable fatty acids of the corresponding series. **C,D:** Arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA). Astrocytes isolated from newborn hamsters were grown in MEM + 5% FCS containing no supplement (vehicle), α-tocopherol alone (70 μM), or DHA (30 or 60 μM) plus α-tocopherol (70 μM). $^{\star}P < .05$, $^{\star\star}P < .01$, significant difference between DHA-supplemented cells and controls (α-tocopherol 70 μM). $^{\#}P < .05$, $^{\#\#}P < .01$ significant difference between 30 μM DHA-supplemented cells and 60 μM DHA-supplemented cells (ANOVA).

only a 14% rise in DHA in PE (comparing cells supplemented with 60 μM DHA vs. 30 μM). This indicates that PE is maximally enriched with the DHA concentrations used, but PC is not.

DHA-supplemented cells also had much more EPA in their membranes. The increase was dose-dependent in PC but not in PE. EPA (20:5n-3) is the n-3 metabolic equivalent of AA (20:4n-6) and can be formed enzymatically by retroconversion of DHA (22:6n-3). Its increase in membrane phospholipids indicates an active enzymatic retroconversion of DHA in supplemented cells. The increases in incorporated DHA and EPA resulted in the proportion of n-3 PUFA in supplemented cells reaching 40% of the total fatty acids in PE and only 14% in control cells. The n-3 PUFA reached 20% to 28% in PC (in cells supplemented with 30 and 60 μM, respectively) and only 4.7% in controls. The incorporation of exogenous DHA into astrocyte membranes led to compensatory decreases in AA in PE and PC. AA was the main n-6 PUFA in all groups of cells. The overall bulk of n-6 PUFA in the PE of DHA-supplemented cells was strongly and dose dependently decreased (−64% in 30 μM supplemented cells and

TABLE I. PUFA Composition in Phosphatidylethanolamine of Cultured Astrocytes and Hamsters Cortex†

Fatty acids in PE (% by weight)	Cultured astrocytes				Hamsters cortex	
	Controls	DHA	AA	DHA + AA	Balanced diet	n-3-Deficient diet
20:3n-9	5.5 ± 0.12[a]	0.6 ± 0.03[b]	0.9 ± 0.12[b]	0.7 ± 0.09[b]	0.1 ± 0.14	0.02 ± 0.02
20:4n-6 (AA)	25.0 ± 1.78[a]	14.9 ± 0.93[b]	38.2 ± 0.85[c]	36.6 ± 0.18[c]	15.9 ± 0.98	14.2 ± 1.66
22:4n-6	1.6 ± 0.1[a]	0.3 ± 0.03[b]	7.1 ± 0.35[c]	2.5 ± 0.03[a]	4.2 ± 0.07	5.5 ± 0.2**
22:5n-6	0.4 ± 0.03[a]	0.03 ± 0.03[b]	2.3 ± 0.06[c]	0.4 ± 0.01[a]	3.1 ± 0.83	11.6 ± 0.77**
Σn-6	28.2 ± 1.85[a]	16.4 ± 1.14[b]	48.4 ± 0.79[c]	40.3 ± 0.33[d]	23.8 ± 0.44	32.3 ± 2.76*
20:5n-3 (EPA)	2.2 ± 0.07[a]	11.2 ± 0.48[b]	0.6 ± 0.09[c]	2.4 ± 0.03[a]	0	0
22:5n-3	4.6 ± 0.33[a]	2.6 ± 0.18[b]	2.6 ± 0.06[b]	2.0 ± 0.03[b]	0.2 ± 0.11	0.03 ± 0.03
22:6n-3 (DHA)	7.6 ± 0.92[a]	22.6 ± 1.07[b]	5.7 ± 0.38[a]	16.6 ± 0.13[c]	24.6 ± 3.12	11.0 ± 3.12**
Σn-3	14.9 ± 1.17[a]	36.8 ± 1.62[b]	9.3 ± 0.49[c]	21.3 ± 0.21[d]	25.6 ± 2.61	11.4 ± 1.16**
n-3 + n-6	43.0 ± 2.99[a]	53.2 ± 2.71[b]	57.7 ± 1.26[b]	57.4 ± 4.30[b]	49.3 ± 2.80	43.7 ± 3.80
n-3/n-6	0.5	2.2	0.2	0.5	1.1	0.4

†Values are mean ± standard error of the mean. Cultured astrocytes: n = 3; values with different letters are significantly different from each other (a ≠ b ≠ c, $p < .01$). Astrocytes were grown in MEM + 5% FCS containing 70 μM α-tocopherol (controls) supplemented with 30 μM DHA (DHA) or with 30 μM AA (AA) or with 15 μM DHA + 15 μM AA (DHA + AA). Hamsters cortex: n = 4; *$P < .05$, **$P < .01$ differences between cortex from hamsters fed the n-3 PUFA-deficient diet and from those fed the n-3/n-6 PUFA-balanced diet. AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PE, phosphatidylethanolamine.

–77% in 60 μM supplemented cells compared with controls). The decrease in n-6 PUFA was less pronounced in PC than in PE (–30% on average in 30 and 60 μM supplemented cells vs. controls).

The increased proportion of n-3 PUFA in DHA-supplemented cells was also offset by a decrease in the proportion of MUFA in PE (–25% on average in DHA-supplemented cells vs. controls) and more notably in PC (–40% on average in DHA-supplemented cells vs. controls). SFA were similar in DHA-supplemented cells and in controls.

Effect of Supplementation With DHA Plus AA on Astrocyte Fatty Acid Composition

Table I compares the changes in the PUFA in PE of astrocytes grown in medium supplemented with DHA or AA alone (30 μM) or with both PUFA (15 μM DHA + 15 μM AA) in similar culture conditions (70 μM α-tocopherol and 5% FCS). The PUFA composition of hamster brain cortex is included in Table I as a physiological reference.

The total n-6 + n-3 PUFA concentrations in all group of supplemented cells (AA, DHA, and AA + DHA) were similar and were significantly higher than in controls. The increased n-3 + n-6 PUFA in supplemented cells was associated with loss of the atypical PUFA 20:3n-9, indicating a lack of essential n-6 and n-3 fatty acids in control cells. 20:3n-9 is produced by enzymatic desaturation of n-9 MUFA when n-3 or n-6 substrates are lacking. Its presence in control cells and its absence from PUFA-supplemented cells indicate that the basal medium containing 5% FCS did not provide sufficient n-3 or n-6 PUFA (see FCS fatty acid composition in Table II). Control cells cultured in 10% FCS still had a significant proportion of 20:3n-9 (1.9% ± 0.1% in PE and 1.1% ± 0.05% in PC; data not shown), indicating that classical basal culture conditions (5% or 10% FCS) do not provide enough n-3/n-6 fatty acids. AA was as efficient as DHA in restoring the natural high content of PUFA in cultured astrocytes.

TABLE II. Fatty Acid Composition of Fetal Calf Serum (FCS) Total Lipids*

Fatty acids	Percentage by weight	Micromolar
Σ Saturated	56.4	
Σ Monounsaturated	16.7	
18:2n-6	5.2	68.9
20:2n-6	0.7	8.4
20:3n-6	0.6	7.1
20:4n-6	7.4	90.4
Σn-6 PUFA	13.9	174.8
18:3n-3	0.8	10.7
20:5n-3	1.7	20.8
22:5n-3	3.9	43.9
22:6n-3	5.2	58.8
Σn-3 PUFA	11.6	134.2
Other fatty acids	1.4	
Σ Total	100	

*The data are expressed in percentage of total fatty acids and in μM in raw serum. PUFA, polyunsaturated fatty acids.

AA supplementation shifted the PUFA equilibrium toward the n-6 derivatives, mainly AA (20:4n-6) but also 22:4n-6 and DPA (22:5n-6), the elongated and unsaturated metabolites of AA. The concentrations of these n-6 PUFA were very low or undetectable in controls and DHA-supplemented cells (DHA cells). Their increase in AA-supplemented cells (AA cells) is reminiscent of that in the cortex of n-3-deficient hamsters. These 22-carbon n-6 derivatives are incorporated to replace DHA. The most unsaturated compound 22:5n-6 was preferentially incorporated in vivo, whereas the simply elongated one, 22:4n-6, predominated in vitro. The concentrations of n-3

PUFA (DHA, EPA, and 22:5n-3) were all significantly lower in AA cells than in controls.

The concentration of AA in AA + DHA supplemented cells (AA + DHA cells) remained as high as in AA cells, but concentrations of 22:4n-6 and 22:5n-6 decreased significantly as they were replaced by n-3 PUFA, mainly DHA. AA was much more elevated in astrocytes cultured in medium containing AA + DHA than in the hamster cortex. The total n-3 PUFA concentration, as with that of DHA, was lower than in DHA-supplemented cells or in the cortex of hamsters fed the n-3/n-6-balanced diet. EPA, which was abundant in DHA cells, fell to control cells levels in DHA + AA cells.

If we compare the n-3/n-6 ratio in the hamster cortex and in cultured astrocytes, cells incubated with AA were severely deficient in n-3 PUFA; control cells and cells incubated in DHA + AA had less pronounced imbalances, whereas cells incubated with DHA were enriched in DHA.

The variations in PC (data not shown) caused by the three medium supplements were generally similar to those observed in PE, except that less DHA was incorporated, and the fraction of EPA was proportionally higher than in PE. The n-3/n-6 ratio in PC was 0.5 for control cells, 1.4 for DHA cells, 0.1 for AA cells, and 0.4 for AA + DHA cells. In comparison, the n-3/n-6 ratio in the cortex of hamsters fed an n-3-deficient diet was 0.2, and the ratio was 0.9 in those fed an n-3/n-6-balanced diet.

Influence of DHA Supplementation on Cell Culture Integrity: Protective Effect Against DHA-Induced Peroxidation by α-Tocopherol

DHA, which contains six double bonds, is most sensitive to oxidation and may cause peroxidation when added to cell cultures. We therefore tested the potentially cytotoxic action of adding DHA to the culture medium and its neutralization by α-tocopherol, a potent lipophilic antioxidant. The protective action of α-tocopherol was evaluated by measuring cell peroxidation (TBARS production) and cell growth (total DNA content) after 1 week in culture.

The peroxidation in our cultures is shown in Figure 3A. Adding DHA alone (30 μM) to the culture medium caused a significant increase in TBARS (+40% on average of that in control cells receiving vehicle only). This increase was totally abolished by adding 45 μM or 70 μM α-tocopherol to the medium. α-Tocopherol alone markedly depressed (–72%) the basal production of TBARS compared with control cells (receiving vehicle only) on culture day 6 but not on day 9.

Cell cultures grown in a high concentration of DHA alone (60 μM) had less DNA than control cultures on days 6 and 8, indicating a slight impairment of cell growth during the exponential proliferation phase (Fig. 3B). This effect did not occur in cells given a lower concentration of DHA (30 μM; data not shown). α-Tocopherol (45 or 70 μM) alone had no effect on cell growth but blocked the inhibitory effect of DHA when added to the medium at the same time. DHA-induced growth impairement was

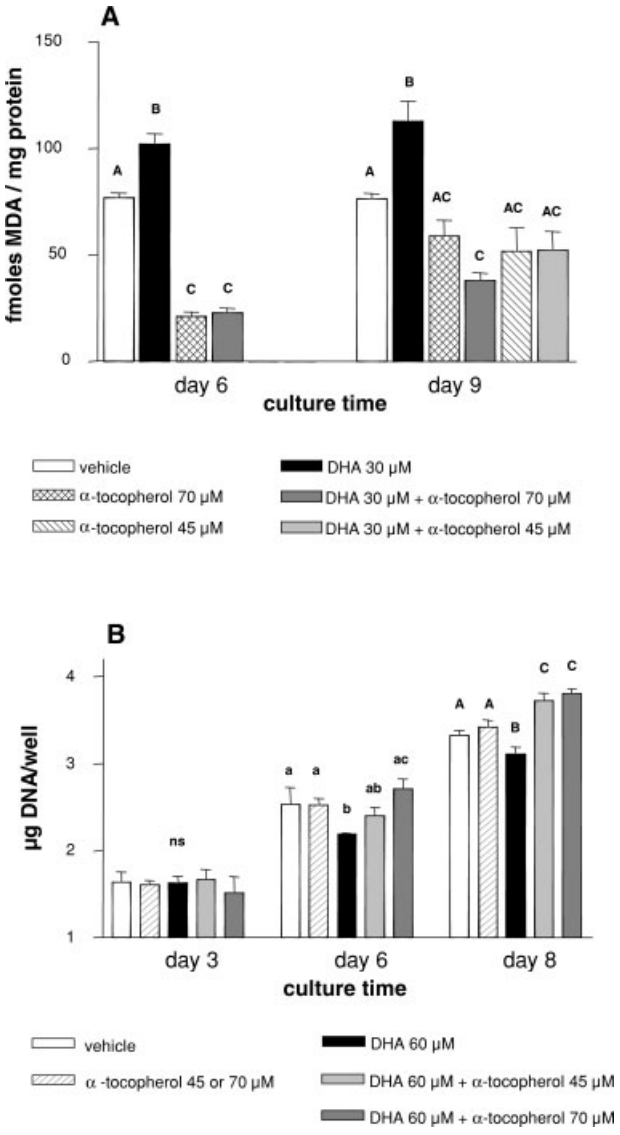

Fig. 3. Protection by α-tocopherol against DHA-induced peroxidation. **A:** Evaluation of cell lipid peroxidation by production of TBARS. **B:** Evaluation of cell growth by measuring the DNA content of the cultures. Astrocytes isolated from newborn hamster cortex were grown in MEM + 5% FCS containing no supplement (vehicle), or α-tocopherol alone (45 or 70 μM), or DHA (30 μM for TBARS production; 60 μM for cell growth) or DHA plus α-tocopherol. Values with different letters are significantly different from each other. a ≠ b ≠ c $P < .05$, A ≠ B ≠ C $P < .01$ (ANOVA).

less well blocked by 45 μM α-tocopherol than by 70 μM when cells were in midgrowth phase (on day 6). We therefore routinely added 70 μM α-tocopherol to medium supplemented with DHA or AA.

Influence of DHA Supplementation on Cell Culture Integrity: Effect on Astrocyte Growth and Differentiation

Cells cultured in medium plus α-tocopherol alone or supplemented with DHA had similar growth patterns,

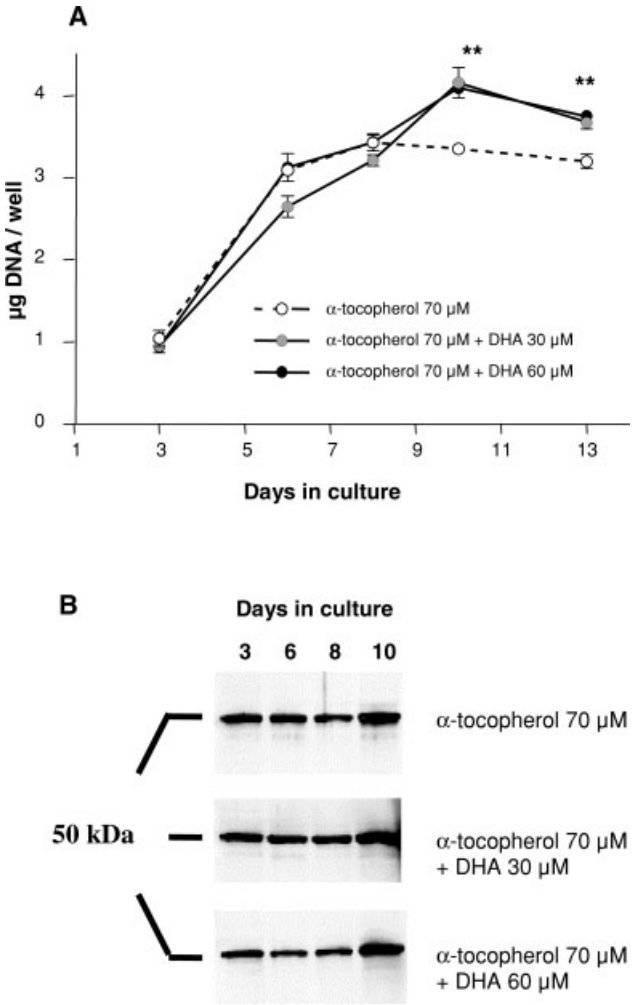

Fig. 4. Effect of DHA supplementation on astrocyte growth and differentiation in culture. **A:** Measurement of DNA in the cultures. **B:** Western blotting of glial fibrillary acidic protein (GFAP). Astrocytes isolated from newborn hamsters cortex were grown in MEM + 5% FCS containing α-tocopherol alone (70 µM) or DHA (30 or 60 µM) plus α-tocopherol 70 µM. $^{**}P < .01$, significant difference vs. cells grown with α-tocopherol alone (70 µM).

with exponential proliferation during the first week, followed by a plateau once confluence was reached (Fig. 4A). However, the proliferative phase lasted longer in DHA-supplemented cells, leading to significantly more cells (+20%) at confluence than in cultures with α-tocopherol alone. This effect was of similar amplitude for cells given 30 and 60 µM DHA.

Western blotting (Fig. 4B) showed that the expression profiles of GFAP in cells supplemented with 30 or 60 µM DHA and in cells given α-tocopherol alone were the same. The intensities of GFAP labeling on days 3, 6, and 8 were similar, with marked increases on day 10. The day-10 increases, indicating the phenotypic maturation of postproliferative astrocytes, were similar in all groups of cells.

DISCUSSION

The n-3/n-6 PUFA profile in the membrane phospholipids of cultured astrocytes can be considerably altered by adding LC-PUFA to the medium. We believe this to be the first study that has analyzed the impact of DHA and AA supplementation on all series of fatty acids, separately in the two main classes of phospholipids, PE and PC, in the membranes of cultured astrocytes.

DHA and AA were effectively incorporated into PE and PC. This confirms and extends previous studies indicating that cultured rat astrocytes actively incorporate DHA from the medium into their lipids (Galli et al., 1992; Williard et al., 2001). Insofar as PE and PC represent, respectively, 36% and 40% of the total phospholipids, this huge increase in LC-PUFA can considerably modify the properties of the cell membrane. The LC-PUFA supplementations led to four types of astrocytes differing mostly in their n-3/n-6 ratio. The n-3/n-6 balance of astrocytes varied over a physiological range comparable to those found in the brain cortex of hamsters fed different diets, but the incorporation of some individual n-3 and n-6 fatty acids differed in vivo and in vitro. Control cells were cultured under classical conditions, using 5% FCS. The fatty acid profiles of cells cultured in medium plus 10% FCS were also analyzed (data not shown) and were similar to those of cells cultured in medium plus 5% FCS. The fatty acid profiles of these cells are clearly evocative of n-3 PUFA deficiency in the PE and PC, with a DHA content lower than that in hamsters fed the n-3-deficient diet. In vivo, as previously shown in other species (Bourre et al., 1984; Guesnet et al., 1988; Clandinin et al., 1992; Ximenes da Silva et al., 2002), the lack of DHA is offset by an increase in DPA (22:5n-6), the n-6 metabolic equivalent of DHA. In contrast, the presence of 2–5% trienoic acid 20:3n-9 in control astrocytes indicates that their PUFA metabolism enzymes used nonspecific n-9 substrates (Innis, 1991), suggesting that the cells did not receive enough n-6 PUFA to compensate totally for their n-3 deficit. Thus, medium containing 5% or 10% FCS does not provide enough PUFA to maintain a physiological incorporation of DHA into membrane phospholipids as well as a physiological compensation of n-6 PUFA. This is in agreement with the findings of Galli et al. (1992) and Murphy et al. (1995) showing high AA levels but decreased DHA levels in rat astrocytes cultured in FCS-containing medium. The n-3/n-6 PUFA ratio in FCS may depend on diet of the donor. Our FCS batch has a well-balanced n-6/n-3 PUFA content (n-6/n-3 ratio = 1.3) and contains intermediate DHA concentration (58.8 µM) compared with other FCS batches previously analyzed in the laboratory (n-6/n-3 ratio = 1.3–3.2; DHA = 28–85 µM; Bardon et al., 1996; Goustard-Langelier et al., 2000). Therefore, many astrocytes cultured in FCS may be even more n-3 deficient than those analyzed in the present study. Hence, in vitro studies have to take into account that most of the astrocytes cultured in FCS medium are DHA deficient, with high AA. In consideration of the high physiological concentration of DHA

in brain cells in vivo, the place of AA in astrocyte signalling should be reevaluated using cultured cells containing physiological amounts of DHA. Petroni et al. (1994) have shown, in line with this, that the production of eicosanoids by DHA-supplemented astrocytes and unsupplemented ones is different.

The amount of DHA in the PE and PC of DHA-supplemented cells was even more elevated than that in the cortex of hamsters fed the n-3/n-6-balanced diet. The main difference between the cultured cells and fresh brain tissue was the presence of EPA, which is usually not found in mammalian brains. Our FCS contained non-negligeable amounts of EPA, so the cells naturally took it up and incorporated it into phospholipids. The large amount of EPA in DHA-supplemented cells also suggests that there is active retroconversion of DHA to EPA. The larger n-3 pool, smaller n-6 pool, and presence of EPA in DHA-supplemented astrocytes is reminiscent of the PUFA profile in brains of mammals fed a diet rich in fish oil, with a low AA concentration and increased DHA (Bourre et al., 1992; Alessandri et al., 1998). However, although a diet supplemented with fish oil or DHA increases the EPA concentration in the blood plasma and several tissues, it does not induce significant incorporation of EPA in the brain (Bourre et al., 1992; Poumès-Ballihaut et al., 2001). This specific exclusion of EPA from brain tissue may involve physiological mechanisms that are not retained in cultured astrocytes.

Astrocytes grown in medium with added AA revealed that very high levels of this n-6 PUFA can be esterified in PE (up to 38% of total fatty acids) and PC (13% of total fatty acids). These concentrations were more than twice those in the cortex from both groups of hamsters. Such high AA levels have nevertheless been found in vivo in the glial cells of young rats fed a diet rich in AA (containing 1% AA; Jumpsen et al., 1997). In vivo, large amounts of DPA (22:5n-6), the n-6 PUFA whose structure is closest to that of DHA, accumulated in the brain phospholipids of n-3-deprived hamsters to replace DHA. Cells grown in AA-enriched medium incorporated little DPA compared with 22:4n-6, indicating that the endoplasmic/peroxisomal pathway (converting 22:4n-6 to 22:5n-6) was not very active.

Growing cells in media enriched with AA and DHA did not reduce the very high AA incorporation resulting from AA supplementation, but it increased DHA incorporation and avoided the EPA incorporation that occurred in cells given DHA alone. The DHA enrichment, nevertheless, remained lower than that in the cortex of hamsters fed an n-3/n-6-balanced diet.

With reference to the fatty acid composition of fresh brain cortex, the results indicate that the physiological PUFA composition cannot be exactly reproduced in cultured astrocytes. It can nevertheless be improved by supplementing the medium with DHA alone or with DHA plus AA. This is in agreement with the work of Bourre et al. (1983) on cultured mixed brain cells. The PUFA profiles in PE suggest that AA is the dominant PUFA in cultured astrocytes. The lower AA content of brain tissue might be because the other cell types in the brain contain less AA than astrocytes. However, analyses of freshly prepared fractions from rat brain performed by Bourre et al. (1984) indicated that astrocytes in situ do not contain more AA than neurons and oligodendrocytes. Thus, the predominance of AA in the astrocytes in vitro is probably a nonphysiological feature of the cultured cells. AA concentrations remained high in cells grown in medium supplemented with both AA and DHA. This suggests that n-6 PUFA are more efficiently captured, metabolized, and/or esterified into phospholipids by cultured astrocytes than are n-3 PUFA. This may be explained by the newborn origin of the cultured cells since n-6 PUFA seem to predominate in the brain PE in early life (Martinez et al., 1974; Jumpsen et al., 1997).

Modulating the PUFA profile of astrocytes directly by adding LC-PUFA (DHA and AA) to the medium has several advantages over essential fatty acid (EFA; linoleic and α-linolenic acids) supplementation. Studies using EFA supplementation in astrocytes have shown that the peroxisomal pathway leading to LC-PUFA synthesis in these cells is not sufficient and that most of the AA and DHA produced are released in the medium (Moore et al., 1991; Williard et al., 2001). Therefore, high non-physiological levels of EFA, as well as PUFA intermediates usually not found in vivo, accumulate in the membranes (Bernoud et al., 1998; Innis and Dyer, 2002).

One potential problem with direct LC-PUFA supplementation is the high peroxidability of these compounds, notably DHA, the most unsaturated PUFA (Visioli et al., 1998). We find that α-tocopherol effectively prevents lipid peroxidation in our cultures. The moderate increase in cell lipid peroxidation products (TBARS) and the slight reduction in cell growth during the proliferative phase caused by adding DHA alone were totally suppressed by adding α-tocopherol to the medium.

DHA supplementation in the presence of α-tocopherol does not appear to alter the main characteristics of the culture. DHA-supplemented cells and unsupplemented cells had similar growth patterns and degrees of astrocyte differentiation, as judged by the expression of GFAP. GFAP increased in both groups of cells after they became confluent, reflecting normal astrocyte phenotypic maturation (Tardy et al., 1990). We find that DHA increased the cell number at confluence, once the cells had ceased to divide, but not during exponential growth. This suggests that DHA reduced cell mortality rather than interfered with cell multiplication. DHA has been shown to protect other cell types from apoptosis, notably, neuronal cells (Kim et al., 2001). Thus, restoring physiological DHA stores in membrane phospholipids appears to improve cell survival in our DHA-supplemented cultures.

In conclusion, we have shown that culturing hamster astrocytes in medium supplemented with LC-PUFA greatly affects their membrane PUFA, producing a range of profiles mimicking, at least partially, physiological vari-

ations found in the brains of hamsters fed diets with different n-3/n-6 PUFA contents. This study emphasizes the difference between the n-3 dominance in the brain and the n-6 dominance in cultured astrocytes and the need to add DHA to the medium to correct this. Giving the importance of membrane motility in astrocytes, the amount of DHA in their membranes may affect key components of the astrocyte syncytium. The sensitive and cognitive elements that are affected in DHA-deficient brains rely in part on astrocyte plasticity. The in vitro manipulations we describe here will be useful for exploring the role of DHA in these cellular mechanisms.

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REFERENCES

Alessandri JM, Goustard B, Guesnet P, Durand G. 1998. Docosahexaenoic acid concentrations in retinal phospholipids of piglets fed an infant formula enriched with long-chain polyunsaturated fatty acids: effects of egg phospholipids and fish oils with different ratios of eicosapentaenoic acid to docosahexaenoic acid. Am J Clin Nutr 67:377–385.

Bardon S, Le MT, Alessandri JM. 1996. Metabolic conversion and growth effects of n-6 and n-3 polyunsaturated fatty acids in the T47D breast cancer cell line. Cancer Lett 99:51–58.

Bernoud N, Fenart L, Bénistant C, Pageaux JF, Dehouck MP, Molière P, Lagarde M, Cecchelli R, Lecerf J. 1998. Astrocytes are mainly responsible for the polyunsaturated fatty acid enrichment in blood-brain barrier endothelial cells in vitro. J Lipid Res 39:1816–1824.

Berry JF, Bovis M, Logothetis J. 1965. Determination of the fatty acid composition of cerebrospinal fluid by gas-liquid chromatography. Neurology 15:1089–1094.

Bourre JM, Faivre A, Dumont O, Nouvelot A, Loudes C, Puymirat J, Tixier-Vidal A. 1983. Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. J Neurochem 41:1234–1242.

Bourre JM, Pascal G, Durand G, Masson M, Dumont O, Piciotti M. 1984. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. J Neurochem 43:342–348.

Bourre JM, François M, Youyou A. 1989. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning task in rats. J Nutr 119:1880–1892.

Bourre JM, Bonneil M, Chaudière J, Clément M, Dumont O, Durand G, Lafont H, Nalbone G, Pascal G, Piciotti M. 1992. Structural and functional importance of dietary polyunsaturated fatty acids in the nervous system. Adv Exp Med Biol 318:211–229.

Broughton KS, Wade JW. 2002. Total fat and (n-3):(n-6) fat ratios influence eicosanoid production in mice. J Nutr 132:88–94.

Carrié I, Smirnova M, Clément M, De Javel D, Frances H, Bourre JM. 2002. Docosahexaenoic acid-rich phospholipid supplementation: effect on behavior, learning ability, and retinal function in control and n-3 polyunsaturated fatty acid deficient old mice. Nutr Neurosci 5:43–52.

Clandinin MT, Suh M, Hargreaves K. 1992. Impact of dietary fatty acid balance on membrane structure and function of neural tissues. Adv Exp Med Biol 318:197–210.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tisues. J Biol Chem 226:507–509.

Galli C, Marangoni F, Petroni A. 1992. Modulation of arachidonic acid metabolism in cultured rat astroglial cells by long-chain n-3 fatty acids. Adv Exp Med Biol 318:115–120.

Giaume C, Marin P, Cordier J, Glowinski J, Premont J. 1991. Adrenergic regulation of intercellular communications between cultured striatal astrocytes from the mouse. Proc Nat Acad Sci USA 88:5577–5581.

Goustard-Langelier B, Alessandri JM, Raguenez G, Durand G, Courtois Y. 2000. Phospholipid incorporation and metabolic conversion of n-3 polyunsaturated fatty acids in the Y79 retinoblastoma cell line. J Neurosci Res 60:678–685.

Guesnet P, Pascal G, Durand G. 1988. Effects of dietary alpha-linolenic acid deficiency during pregnancy and lactation on lipid fatty acid composition of liver and serum in the rat. Reprod Nutr Dev 28:275–292.

Holman RT, Johnson SB, Hatch TF. 1982. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 35:617–623.

Innis SM. 1991. Essential fatty acids in growth and development. Prog Lipid Res 30:39–103.

Innis SM, Dyer RA. 2002. Brain astrocyte synthesis of docosahexaenoic acid from n-3 fatty acids is limited at the elongation of docosapentaenoic acid. J Lipid Res 43:1529–1536.

Jumpsen J, Lien EL, Goh YK, Clandinin MT. 1997. Small changes of dietary (n-6) and (n-3) fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. J Nutr 127:724–731.

Kim HY, Akbar M, Kim KY. 2001. Inhibition of neuronal apoptosis by polyunsaturated fatty acids. J Mol Neurosci 16:223–227.

Labarca C, Paigen K. 1980. A simple, rapid, and sensitive DNA assay procedure. Anal Biochem 102:344–352.

Litman BJ, Mitchell DC.1996. A role for phospholipid polyunsaturation in modulating membrane protein function. Lipids 31:S193–S197.

Martinez AD, Saez JC. 1999. Arachidonic acid-induced dye uncoupling in rat cortical astrocytes is mediated by arachidonic acid byproducts. Brain Res 816:411–423.

Martinez M, Conde C, Ballabriga A. 1974. Some chemical aspects of human brain development: II. Phosphoglyceride fatty acids. Pediatr Res 8:93–102.

Moore SA, Yoder E, Murphy S, Dutton GR, Spector AA. 1991. Astrocytes, not neurons, produce docosahexaenoic acid (22:6ω-3) and arachidonic acid (20:4ω-6). J Neurochem 56:518–524.

Moriguchi T, Greiner RS, Salem N Jr. 2000. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem 75:2563–2573.

Morrison WR, Smith LM. 1964. Preparation of fatty methylesters and dimethylacetals from lipids with boron fluoride methanol. J Lipid Res 5:600–608.

Murphy MG. 1995. Effects of exogenous linoleic acid on fatty acid composition, receptor-mediated cAMP formation, and transport functions in rat astrocytes in primary culture. Neurochem Res 20:1365–1375.

Petroni A, Salami M, Blasevich M, Papini N, Galli C. 1994. Inhibition by n-3 fatty acids of arachidonic acid metabolism in a primary culture of astroglial cells. Neurochem Res 19:1187–1193.

Pietsch A, Lorenz R. 1993. Rapid separation of the major phospholipid classes on a single aminopropyl cartridge. Lipids 28:945–947.

Poumès-Ballihaut C, Langelier B, Houlier F, Alessandri JM, Durand G, Latge C, Guesnet P. 2001. Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. Lipids 36:793–800.

Salem N Jr, Litman B, Kim HY, Gawrisch K. 2001. Mechanism of action of docosahexaenoic acid in the nervous system. Lipids 36:945–959.

Sanchez-Olea R, Morales-Mulia M, Moran J, Pasantes-Morales H. 1995. Inhibition by polyunsaturated fatty acids of cell volume regulation and osmolyte fluxes in astrocytes. Am J Physiol 269:C96–C102.

Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP. 1995. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J Lipid Res 36:2471–2477.

Tardy M, Fages C, Le Prince G, Rolland B, Nunez J. 1990. Regulation of the glial fibrillary acidic protein (GFAP) and of its encoding mRNA in the developing brain and in cultured astrocytes. Adv Exp Med Biol 265:41–52.

Uauy R, Mena P. 2001. Lipids and neurodevelopment. Nutr Rev 59:S34–S46.

Visioli F, Colombo C, Galli C. 1998. Oxidation of individual fatty acids yields different profiles of oxidation markers. Biochem Biophys Res Commun 245:487–489.

Wainwright PE. 1992. Do essential fatty acids play a role in brain and behavioral development? Neurosci Biobehav Rev 16:193–205.

Wallin B, Rosengren B, Shertzer HG, Camejo G. 1993. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtitre plate: its use for evaluation of antioxidants. Anal Biochem 208:10–15.

Williard DE, Harmon SD, Kaduce TL, Preuss M, Moore SA, Robbins MEC, Spector AA. 2001. Docosahexaenoic acid synthesis from n-3 polyunsaturated fatty acids in differenciated rat brain astrocytes. J Lipid Res 42:1368–1376.

Ximenes da Silva A, Lavialle F, Gendrot G, Guesnet P, Alessandri JM, Lavialle M. 2002. Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. J Neurochem 81:1328–1337.

Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI. 1998. Fatty acids and brain peptides. Peptides 19:407–419.

Yu ACH, Chan PH, Fishman RA. 1986. Effects of arachidonic acid on glutamate and d-aminobutyric acid uptake in primary cultures of rat cerebral cortical astrocytes and neurons. J Neurochem 47:1181–1189.

Yu N, Martin JL, Stella N, Magistretti PJ. 1993. Arachidonic acid stimulates glucose uptake in cerebral cortical astrocytes. Proc Nat Acad Sci USA 90:4042–4046.