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Correlation of Taurine Transport with Membrane Lipid Composition and Peroxidation in DHA-Enriched Caco-2 Cells

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Abstract Diets supplemented with n-3 polyunsaturated fatty acids can promote lipid peroxidation and the propagation of oxygen radicals. These effects can be prevented by taurine, a functional ingredient with antioxidant properties. Here, we examined whether there is a correlation between transepithelial taurine transport, on the one hand, and membrane fatty acid composition and peroxidation in intestinal Caco-2 cells, on the other. Differentiated Caco-2 cells were maintained for 10 days, from the day of confluence, in control conditions or in a medium enriched with docosahexaenoic acid (DHA, 100 μmol/l), taurine (10 mmol/l) or DHA plus taurine. Incubation of the monolayers in a medium enriched with DHA increased the incorporation of this fatty acid into the brush-border membrane, at the expense of total n-6 fatty acids (C20:2n-6, C20:3n-6 and C22:4n-6). This was paralleled by increased membrane lipid peroxidation, which was partially limited by the addition of taurine. Transepithelial taurine transport was estimated from taurine uptake and efflux kinetic parameters at apical and basolateral domains. Cell incubation with DHA increased basolateral taurine uptake through an increase in V_{max}, whereas incubation with taurine downregulated basolateral uptake as occurred for apical taurine transporter. Moreover, addition of DHA reduced the apical downregulation effect exerted on taurine transport by taurine incubation. Our results suggest that the oxidative status of epithelial cells regulates taurine transport, thus satisfying antioxidant cellular requirements.

Keywords TAUT · Transeptihelial transport · Kinetic characterization · n-3 and n-6 polyunsaturated fatty acids · Oxidative stress

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Introduction

Docosahexaenoic acid (DHA, C22:6n-3) is involved in many important physiological functions during postnatal development, especially in brain, retina, heart and blood (Moghadasian 2008). For this reason, dietary supplementation with DHA is increasingly common. Nevertheless, a high intake of polyunsaturated fatty acids (PUFAs) can also be deleterious as it may promote lipid peroxidation and the subsequent propagation of oxygen radicals, which are involved in the pathogenesis of various gastrointestinal disorders (Kaplan et al. 2007; Naito and Yoshikawa 2006). In a previous study (Roig-Pérez et al. 2004) we demonstrated that Caco-2 cell supplementation with DHA increased lipid peroxidation and paracellular permeability, in parallel with a redistribution of the tight junction proteins occludin and ZO-1. This effect was, in part, mediated by hydrogen peroxide and peroxynitrite and was prevented by the addition of taurine, an amino acid that counteracts the effects of hydrogen peroxide.

Taurine is the most abundant intracellular amino acid in humans and is required for a number of biological processes, including osmoregulation, antioxidation and detoxification (Bouckenooghe et al. 2006; Huxtable 1992). Taurine concentration is notably high (up to the millimolar range) in tissues subjected to oxidative stress such as brain,

retina, heart, lung and intestine (Huxtable 1992; Wersinger et al. 2001). In the intestine, taurine is transported across the apical membrane by system TAUT, a Na^+- and Cl^--dependent transport mechanism that is specific to β-amino acids (Roig-Pérez et al. 2005). In a previous study, we characterized transepithelial taurine transport in Caco-2 cell monolayers and found that basolateral taurine uptake is mediated by a transport mechanism with many of the properties of apical system TAUT (Roig-Pérez et al. 2005). We also observed that taurine apical influx is higher than basolateral efflux rates, thereby enabling epithelial cells to accumulate this amino acid.

Intestinal epithelial cells are continually exposed to changes in dietary composition. Small alterations in dietary fatty acid contents can modify brush-border membrane fatty acid composition and, consequently, its fluidity properties and function (Thomson et al. 2003; Vázquez et al. 2000). This in turn can modify nutrient absorption (Ferrer et al. 2003; Drozdowski and Thomson 2006). Here, we studied transepithelial taurine transport in Caco-2 cells maintained in DHA-enriched conditions with the aim of establishing whether there is a correlation between such transport and membrane fatty acid composition and lipid peroxidation.

Materials and Methods

Materials

Dulbecco's modified Eagle medium (DMEM), nonessential amino acids, penicillin, streptomycin, L-glutamine, fetal bovine serum (FBS), bovine serum albumin (BSA), D-glucose, taurine (cell culture–tested), DHA, butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO), tris(hydroxymethyl)amino methane (Tris), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) as well as other chemicals were supplied by Sigma (St. Louis, MO). Diphenyl-1-pyrenylphosphine (DPPP) was purchased from Molecular Probes (Eugene, OR). [1,2-^{14}C]Taurine (specific activity 110 mCi/mmol) was from ARC (St. Louis, MO). Tissue culture supplies, including Transwells, were obtained from Costar (Cambridge, MA).

Cell Culture

Caco-2 cells were kindly provided by Dr. David Thwaites from the School of Cell and Molecular Biosciences, University of Newcastle upon Tyne (UK). The cells (passages 107–116) were routinely grown in plastic flasks at a density of $5 \times 10^4/cm^2$ and cultured in DMEM supplemented with 4.5 g/l D-glucose, 1% (v/v) nonessential amino acids, 2 mmol/l L-glutamine, 10% (v/v) heat-inactivated FBS, penicillin 100 U/ml and streptomycin 100 μg/ml at 37°C in a modified atmosphere of 5% CO_2 in air. After reaching confluence (day 6–7 after seeding), cells were grown for 10 additional days in control conditions or in medium supplemented with either DHA (100 μmol/l), taurine (10 mmol/l) or DHA plus taurine (100 μmol/l and 10 mmol/l, respectively). DHA was solubilized in the culture medium, bound to albumin, as previously described (Roig-Pérez et al. 2004). The fatty acid was dissolved in ethanol (containing 4 mg/l BHT) and added, under constant stirring at 56°C, to DMEM containing FBS to achieve a final 3:1 (mol/mol) fatty acid–albumin ratio. The albumin-bound fatty acid solution was filter-sterilized by passage through a 0.2-μm membrane filter before adjusting the volume with complete DMEM to the final desired DHA concentration (100 μmol/l). Control and taurine-maintained cells were exposed to the same final BHT and ethanol concentration as DHA-supplemented medium. The concentration of DHA used in these experiments, although lower than the concentration considered to be physiological (800 μmol/l) (Wang et al. 2001), is the one usually tested in similar fatty acid–enrichment experiments in Caco-2 cells (Dias et al. 1991; Nano et al. 2003; Usami et al. 2001, 2003). The effects of DHA and taurine enrichment on cell viability are reported elsewhere (Roig-Pérez et al. 2004).

Growth medium was replaced twice a week and the day before the experiment. For transport experiments, cells were seeded at a density of $4 \times 10^5/cm^2$ onto polycarbonate filters (Transwells, 12 mm diameter) with a pore size of 0.4 μm.

Membrane Fatty Acid Composition

Studies on fatty acid composition were carried out on brush-border membrane vesicles prepared using the Mg^{2+} precipitation method, as previously described (Ferrer et al. 2003). Vesicles were diluted to a final protein concentration of 20–30 g/l and then frozen and stored in liquid nitrogen in 150-μl aliquots. Each isolation batch corresponded to 2 g of cells, and in "Results" "*n*" indicates the number of isolation batches. The purity of the membrane preparations, evaluated from sucrase activity (Dahlqvist 1964), revealed an 11-fold enrichment factor. Protein content was determined using the Bio-Rad (Richmond, CA) protein assay, with BSA as standard.

Total lipids were extracted from brush-border membranes as previously described (Ferrer et al. 2003). Fatty acid methyl esters (FAMEs, 2 μl of samples) were injected into a Hewlett-Packard (Wilmington, DE) Series II 5890 gas chromatograph (FID detector), equipped with a glass precolumn (2.5 m × 0.25 mm i.d.) coated with deactivated cyanopropyl/phenyl/methyl silicone and a glass capillary column (50 m × 0.25 mm i.d.) coated with 0.2 mm of

stationary phase of 100% cyanopropyl silicone (CP Sil 88; Chrompack, Middleburg, the Netherlands). The oven temperature was programmed as follows: 177°C for 11.3 min, then raised to 235°C at a rate of 2°C/min and maintained for an additional 15 min. Helium was used as carrier gas at a flow rate of 1 ml/min. FAMEs were detected using electron impact ionization with an ion source temperature of 200°C. The same extractive and analytical procedures were applied to the study of the fatty acid composition of the incubation media. Fatty acids were quantified by applying relative response factors, and the results were expressed as compensated area normalization (Guardiola et al. 1994).

Lipid Peroxidation of the Cell Membrane

Cell membrane oxidation was determined using the DPPP assay described by Takahashi et al. (2001). DPPP stoichiometrically reduces H_2O_2 and biologically important hydroperoxides to their corresponding alcohols. DPPP itself is not fluorescent, but DPPP oxide, the resulting product of the reaction with hydroperoxides, is.

Cells were collected, washed three times with PBS and preincubated in PBS at a density of 1×10^8/ml at 37°C for 5 min. After addition of DPPP (solubilized in DMSO and stored in nitrogen atmosphere at –20°C) to a final concentration of 167 μmo/l, the cell suspension was incubated at 37°C for 5 min in the dark. Cells were washed three times with PBS and resuspended in PBS at a density of 3×10^6/ml. The cell suspension was then maintained for 30 min in a CO_2 incubator at 37°C. During the complete labeling procedure, the cell suspension was handled in shaded tubes and kept in the dark. Fluorescence intensity of the samples was measured with the Luminescence spectrometer LS50B (F-2000; Hitachi, Tokyo, Japan) with excitation and emission wavelengths of 351 and 380 nm, respectively.

Uptake Experiments

Transport experiments were performed as previously described (Roig-Pérez et al. 2005). Monolayers grown in filters were gently washed by sequential transfer through four beakers containing 500 ml of modified Krebs buffer at room temperature. The composition of Krebs was (mmol/l) NaCl 137, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.0, NaH_2PO_4 0.3, D-glucose 10 and HEPES/Tris 10 (pH 7.4). The filters were then placed in culture wells containing 1.5 and 0.75 ml modified Krebs buffer in the basal and apical compartments, respectively; and transepithelial electrical resistance (TER) was determined using a Millicell-ERS voltohmmeter (Millipore, Bedford, MA). After TER determination, apical or basolateral medium was replaced by the same volume of modified Krebs containing 0.2 μCi/ml [1,2-^{14}C]taurine and the appropriate unlabeled taurine concentration to study either apical or basolateral uptake. In some experiments NaCl was replaced by an equimolar concentration of KNO_3. After the incubation period, the filters were washed four times in 500 ml ice-cold modified Krebs to eliminate nonspecific radioactivity fixation, removed from the insert and dissolved in scintillation cocktail to be counted in a Packard (Downers Grove, IL) 1500 Tri-Carb counter. At the end of the experiment, a sample of the basal or apical medium from either apical or basolateral substrate incubation, respectively, was also taken for radioactivity quantification. Intracellular taurine concentration was calculated assuming a monolayer volume of 2.14 μl by multiplying the surface area of the filter by the height of the monolayer estimated in transmission electron microscopy micrographs of known magnification (1.89 ± 0.8 μm, mean ± SEM of $n = 20$ micrographs) (Roig-Pérez et al. 2005).

Efflux Experiments

Taurine efflux from the cells was determined as previously described (Roig-Pérez et al. 2005). Briefly, cell monolayers were loaded for 1 h at 37°C with 0.2 μCi/ml [1,2-^{14}C]taurine and the appropriate unlabeled taurine concentration from either the apical or basolateral side for basolateral or apical efflux experiments, respectively. The preloaded monolayers were then washed and placed in culture wells containing modified Krebs buffer in the apical and basolateral compartments. The amount of taurine released was determined by measuring radioactivity in aliquots from apical and basolateral media. Intracellular taurine concentration attained after the loading period was determined in at least three monolayers for each substrate concentration processed as in uptake experiments.

Kinetic and Statistical Analyses

To estimate the kinetic parameters of apical and basolateral uptake, the rates of mediated transport were analyzed as previously described (Roig-Pérez et al. 2005), assuming either a one-system or a two-system model by nonlinear regression from plots generated by the Enzfitter statistical package (Biosoft, Cambridge, UK). The best fit was assigned to the fit showing the lowest as well as significantly different residual sums of squares ($P < 0.05$), following the criteria of Motulsky and Ransnas (1987). In the case of apical and basolateral taurine efflux, the data were analyzed in the same way but assuming either a simple diffusion mechanism, a one-system plus diffusion or a two-system plus diffusion model.

Results are given as mean ± SEM. One-way analysis of variance was followed by Student's *t*- or Scheffe's multiple

comparison test using the SPSS statistical software package, version 14.0 (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered to denote significance.

Transepithelial Taurine Transport

Transepithelial taurine transport was estimated following Chen et al. (1994) and as previously described (Roig-Pérez et al. 2005). In this model, net apical uptake ($J_{net,u,AP}$) is considered to be equal to apical uptake minus apical efflux, as shown in Eq. 1:

$$\begin{aligned} J_{\mathbf{net},u,AP} &= J_{u,AP} - J_{e,AP} \\ &= \frac{V_{max,u,AP} \cdot C_0}{K_{m,u,AP} + C_0} + K_{D,u,AP}(C_0 - C_i) \\ &\quad - K_{D,e,AP}(C_i - C_0) \end{aligned} \tag{1}$$

in which $V_{max,u,AP}$ and $K_{m,u,AP}$ are the kinetic parameters of mediated apical taurine uptake and $K_{D,u,AP}$ and $K_{D,e,AP}$, the apical passive diffusion constants of taurine nonmediated uptake and efflux, respectively. C_0 is apical taurine concentration and C_i, the intracellular concentration achieved at C_0. Similarly, basolateral efflux is considered to be equal to basolateral efflux minus basolateral uptake:

$$\begin{aligned} J_{\mathbf{net},e,BL} &= J_{e,BL} - J_{u,BL} \\ &= K_{D,e,BL}(C_i - C_r) \\ &\quad - \left[\frac{V_{max,u,BL} \cdot C_r}{K_{m,u,BL} + C_r} + K_{D,u,BL}(C_r - C_i)\right] \end{aligned} \tag{2}$$

in which $V_{max,u,BL}$ and $K_{m,u,BL}$ are the kinetic constants of mediated basolateral taurine uptake and $K_{D,e,BL}$ and $K_{D,u,BL}$, the basolateral passive diffusion constants of nonmediated taurine efflux and uptake, respectively. C_i is the intracellular concentration attained as a result of monolayer incubation with C_0 present in the apical compartment, and C_r, which stands for the receiver concentration, is the taurine concentration attained in the basolateral compartment at the end of the 5-min incubation.

Results

Fatty Acid Membrane Composition

The fatty acid composition of the incubation media and brush-border membranes is shown in Table 1. DHA-supplemented medium showed an increase in the percent of this fatty acid without any effect on total n-3 PUFAs or on the proportion of the other fatty acids. Regarding membrane composition, DHA enrichment significantly increased the incorporation of this fatty acid and of C20:4n-3 and, therefore, of total n-3 PUFA content, although a reduction in C18:3n-3 was also detected. The increase in n-3 fatty acids was at the expense of n-6 PUFAs (C20:2n-6, C20:3n-6 and C22:4n-6), despite the increase in C18:2n-6. Moreover, DHA-enriched membranes showed higher C16:0 and C17:0 incorporation, whereas a reduction in chain elongation products C22:0 and C24:0 and in C18:1n-7 was observed. Total saturated and monounsaturated fatty acids (SFAs and MUFAs) and the ratio of PUFAs to SFAs was not modified; however, the proportion n-6/n-3 was significantly reduced.

Effect of DHA and Taurine Enrichment on Membrane Lipid Peroxidation

Membrane lipid peroxidation, evaluated from DPPP labeling, revealed an increase in fluorescence intensity in DHA-incubated cells. This increase was partially limited by the addition of taurine (Fig. 1).

Effect of DHA and Taurine Enrichment on Apical Taurine Uptake

The effect of DHA and taurine enrichment on apical taurine uptake is shown in Fig. 2a. The kinetic parameters (Table 2) were calculated from initial taurine uptake at a range of substrate concentrations (3–100 μmol/l). The nonmediated component was estimated in Na^+- and Cl^--free conditions (NaCl replaced by KNO_3) and subtracted from total taurine uptake. The best fit for the Na^+-dependent transport component, in all the conditions tested, was obtained by considering a model of a single transport system. Incubation with DHA did not modify the kinetics of mediated and nonmediated taurine uptake. In contrast, incubation with this amino acid led to a ninefold increase in K_m without any changes in V_{max} values. The addition of DHA to taurine-enriched medium partially reversed K_m changes (6.5-fold vs. control) and induced an increase in V_{max} values. Nonmediated apical taurine uptake (K_D) was also reduced to a similar extent in all the conditions containing taurine.

Effect of DHA and Taurine Enrichment on Basolateral Taurine Uptake

The kinetic parameters for basolateral uptake were calculated in the same way as for apical transport (Table 3). The best fit for the Na^+-dependent transport component, in all the conditions tested, was also obtained by considering a single transport system (Fig. 2b). DHA enrichment induced an increase in V_{max} with no effect on K_m values. Cells maintained in media enriched with taurine and DHA plus taurine showed an increase in K_m without changes in V_{max} values. Nonmediated basolateral taurine uptake was again reduced in all the conditions containing the amino acid.

Table 1 Fatty acid composition of the incubation medium and brush-border membrane of Caco-2 cells maintained in control or in DHA-enriched conditions

	Incubation medium		Brush-border membrane	
	Control	DHA	Control	DHA
C14:0	1.60 ± 0.02	1.20 ± 0.13	1.24 ± 0.20	1.45 ± 0.03
C15:0	0.64 ± 0.01	0.51 ± 0.01	0.29 ± 0.02	0.61 ± 0.12
C16:0	9.65 ± 0.07	11.82 ± 0.11	11.3 ± 0.10	13.1 ± 0.26*
C17:0	1.59 ± 0.04	1.16 ± 0.02	0.53 ± 0.03	1.26 ± 0.04*
C18:0	4.19 ± 0.03	3.97 ± 0.09	3.73 ± 0.36	2.62 ± 0.35
C19:0	0.50 ± 0.02	0.40 ± 0.03	0.26 ± 0.13	0.21 ± 0.02
C20:0	0.71 ± 0.13	0.43 ± 0.04	0.77 ± 0.20	0.74 ± 0.08
C22:0	0.82 ± 0.06	0.63 ± 0.04	1.05 ± 0.04	0.41 ± 0.05*
C24:0	0.24 ± 0.01	0.18 ± 0.03	0.54 ± 0.04	0.20 ± 0.02*
C14:1n-9	1.20 ± 0.08	1.06 ± 0.02	0.93 ± 0.22	0.97 ± 0.08
C16:1n-9	1.66 ± 0.05	1.45 ± 0.03	0.90 ± 0.44	1.18 ± 0.06
C16:1n-7	0.61 ± 0.01	0.68 ± 0.00	1.12 ± 0.34	0.75 ± 0.06
C18:1n-9	12.74 ± 0.00	12.73 ± 0.06	12.2 ± 0.08	12.3 ± 0.13
C18:1n-7	0.83 ± 0.02	0.93 ± 0.02	1.67 ± 0.12	1.21 ± 0.04*
C20:1n-9	0.73 ± 0.09	0.58 ± 0.01	0.37 ± 0.05	0.60 ± 0.13
C22:1n-9	1.39 ± 0.07	0.84 ± 0.20	0.82 ± 0.34	1.22 ± 0.12
C24:1n-9	0.16 ± 0.01	0.11 ± 0.01	0.18 ± 0.04	0.21 ± 0.01
C18:2n-6	13.64 ± 0.01	17.46 ± 0.38	12.7 ± 0.14	14.0 ± 0.11*
C18:3n-6	0.25 ± 0.08	0.14 ± 0.01	0.33 ± 0.08	0.12 ± 0.09
C20:2n-6	11.66 ± 0.38	10.20 ± 0.03	11.9 ± 0.15	8.61 ± 0.11*
C20:3n-6	10.41 ± 0.17	8.69 ± 0.13	11.0 ± 0.16	7.49 ± 0.15*
C20:4n-6	7.48 ± 0.05	6.37 ± 0.06	7.29 ± 0.31	6.58 ± 0.01
C22:4n-6	1.92 ± 0.03	1.67 ± 0.02	2.43 ± 0.10	1.59 ± 0.06*
C22:5n-6	ND	ND	0.65 ± 0.24	0.15 ± 0.02
C18:3n-3	8.54 ± 0.37	7.67 ± 0.08	9.34 ± 0.06	7.46 ± 0.28*
C18:4n-3	0.56 ± 0.02	0.42 ± 0.03	0.71 ± 0.13	0.68 ± 0.24
C20:4n-3	0.35 ± 0.02	0.27 ± 0.01	0.51 ± 0.05	2.35 ± 0.03*
C20:5n-3	4.36 ± 0.01	3.76 ± 0.14	4.36 ± 0.24	3.72 ± 0.08
C22:5n-3	0.46 ± 0.02	ND	0.50 ± 0.15	0.39 ± 0.05
C22:6n-3	1.10 ± 0.06	4.65 ± 0.08*	0.48 ± 0.08	7.81 ± 0.11*
SFA	19.95 ± 0.30	20.3 ± 0.07	19.7 ± 0.04	20.5 ± 0.14
MUFA	19.3 ± 0.18	18.4 ± 0.21	18.1 ± 0.24	18.3 ± 0.13
n-6 PUFA	45.36 ± 0.07	44.53 ± 0.15	46.2 ± 0.43	38.6 ± 0.31*
n-3 PUFA	15.4 ± 0.40	16.8 ± 0.03	15.8 ± 0.26	22.4 ± 0.07*
n-6/n-3	2.94 ± 0.07	2.65 ± 0.01	2.91 ± 0.05	1.72 ± 0.01*
P/S	4.01 ± 0.08	3.93 ± 0.02	4.07 ± 0.01	3.86 ± 0.03

The incubation medium was enriched with 100 µmol/l DHA. Fatty acids were quantified by applying relative response factors, and the results were expressed as compensated area normalization. Values are mean ± SEM, $n = 6$

* Significant differences ($P < 0.05$) between control and DHA conditions for the incubation medium and for brush-border membranes

ND not detected; *P/S* ratio of polyunsaturated to saturated fatty acids

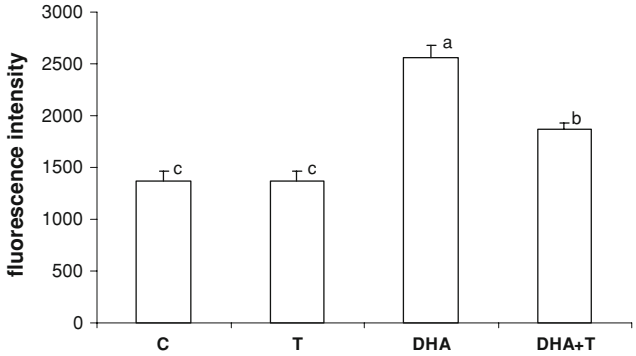

Fig. 1 Fluorescence intensity of DPPP oxide in Caco-2 cell cultures maintained in control conditions (C) or in media enriched with DHA (100 µmol/l), taurine (T, 10 mmol/l) or DHA plus taurine (DHA + T, 100 µmol/l and 10 mmol/l, respectively). Results are expressed as mean ± SEM of $n = 3$ cultures. Mean values with different letters are significantly different ($P < 0.05$)

Effect of DHA and Taurine Enrichment on Apical and Basolateral Taurine Efflux

Cell monolayers were preloaded with increasing taurine concentrations (10–2,000 µmol/l) from the apical side to determine basolateral efflux and from the basolateral side to determine apical efflux. Kinetic analysis of taurine efflux from preloaded cells, taking into account the attained intracellular taurine concentrations, showed the best fit for the two membrane domains and the distinct incubation conditions, by considering a simple diffusion component (data not shown). Significantly higher K_D values for both membrane domains were observed when taurine was present (Tables 2 and 3).

Transepithelial Taurine Transport

To calculate transepithelial taurine transport from the equations shown in "Materials and Methods," the intracellular (C_i) and receiver (C_r) taurine concentrations attained after a 5-min incubation in the presence of a range of substrate concentrations in the apical compartment (C_0) was first considered. After 5-min incubation, taurine did not accumulate in the cells against a concentration gradient (Table 4). Therefore, $C_0 > C_i$ and Eq. 1 of net apical uptake can be simplified to

$$J_{\mathbf{net},u,AP} = J_{u,AP} - J_{e,AP} = \frac{V_{max,u,AP} \cdot C_0}{K_{m,u,AP} + C_0} + K_{D,u,AP}(C_0 - C_i) \quad (3)$$

Moreover, C_r values in the presence of a range of apical substrate concentrations (C_0) were very low (in the

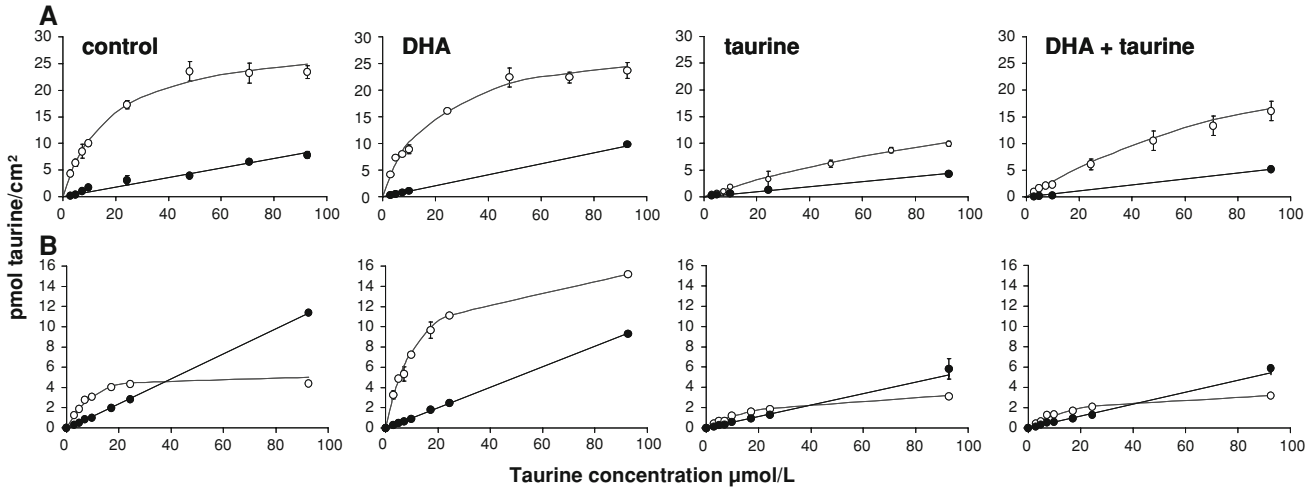

Fig. 2 Substrate concentration dependence of taurine uptake across apical (**a**) and basolateral (**b**) membranes of Caco-2 cell cultures maintained in control conditions or in media enriched with DHA (100 µmol/l), taurine (10 mmol/l) or DHA plus taurine (100 µmol/l and 10 mmol/l, respectively). Monolayers were incubated for 5 min in the presence of increasing unlabeled taurine concentrations (3–100 µmol/l) in the apical or basolateral compartment. The nonmediated component (●) was estimated under Na^+- and Cl^--free conditions. In all the conditions tested, the best fit for the Na^+-dependent component (○) was obtained by considering a model of a single transport system. Results are expressed as mean ± SEM of $n = 3$ monolayers. All these experiments were conducted in parallel, although the data obtained in control conditions were reported elsewhere (Roig-Pérez et al. 2005)

Table 2 Kinetic constants for taurine uptake and efflux across the apical membrane of Caco-2 cell cultures maintained in control conditions or in media enriched with DHA (100 µmol/l), taurine (10 mmol/l) or DHA plus taurine (100 µmol/l and 10 mmol/l, respectively)

	K_m uptake (µmol/l)	V_{max} uptake (pmol/cm^2 · 5 min)	K_D uptake (nl/cm^2 · 5 min)	K_D efflux (nl/cm^2 · 5 min)
Control	17.1 ± 0.80^a	28.4 ± 0.80^a	89.2 ± 9.98^a	72.7 ± 3.31^a
DHA	18.1 ± 2.92^a	29.2 ± 2.82^a	97.3 ± 1.31^a	100 ± 21.5^a
Taurine	154 ± 0.47^b	27.2 ± 0.07^a	51.2 ± 0.34^b	404 ± 86.9^b
DHA + taurine	110 ± 0.22^c	35.8 ± 0.06^b	58.5 ± 6.10^b	346 ± 10.1^b

Kinetic constants were estimated as described in "Materials and Methods." Results are expressed as mean ± SEM of $n = 3$ monolayers. Mean values bearing different superscripts are significantly different ($P < 0.05$)

Table 3 Kinetic constants for taurine uptake and efflux across the basolateral membrane of Caco-2 cell cultures maintained in control conditions or in media enriched with DHA (100 µmol/l), taurine (10 mmol/l) or DHA plus taurine (100 µmol/l and 10 mmol/l, respectively)

	K_m uptake (µmol/l)	V_{max} uptake (pmol/cm^2 · 5 min)	K_D uptake (nl/cm^2 · 5 min)	K_D efflux (nl/cm^2 · 5 min)
Control	9.46 ± 0.60^a	5.59 ± 0.30^a	114 ± 10.6^a	50.1 ± 5.40^a
DHA	13.5 ± 0.37^a	17.8 ± 0.32^b	94.3 ± 3.45^a	44.5 ± 4.20^a
Taurine	20.1 ± 0.60^b	3.52 ± 0.78^a	63.9 ± 5.03^b	310 ± 6.10^b
DHA + taurine	24.1 ± 1.13^b	4.30 ± 0.15^a	61.9 ± 3.47^b	300 ± 14.4^b

Kinetic constants were estimated as described in "Materials and Methods." Results are expressed as mean ± SEM of $n = 3$ monolayers. Mean values bearing different superscripts are significantly different ($P < 0.05$)

nanomoles per liter range, Table 4). Therefore, $C_i > C_r$ and Eq. 2 of net basolateral efflux can be simplified to

$$J_{\mathbf{net},e,BL} = J_{e,BL} - J_{u,BL} = K_{D,e,BL}(C_i - C_r) - \frac{V_{max,u,BL} \cdot C_r}{K_{m,u,BL} + C_r} \quad (4)$$

The results of net apical uptake and basolateral efflux calculated from Eqs. 3 and 4, taking into account the kinetic parameters of Tables 2 and 3 and C_i and C_r values of Table 4, are also shown in Table 4. Our data indicate that estimated net apical uptake was, in all the conditions tested, higher than net basolateral efflux. This difference was more pronounced in DHA-enriched conditions, in which basolateral efflux was lower as a result of the increase in the capacity of basolateral uptake. In taurine-enriched conditions, apical and basolateral downregulation,

Table 4 Intracellular and receiver taurine concentration and estimated net apical taurine influx and efflux rates in Caco-2 cell cultures maintained in control conditions or in media enriched with DHA (100 µmol/l), taurine (10 mmol/l) or DHA plus taurine (100 µmol/l and 10 mmol/l, respectively)

C_0 (µmol/l)	C_i (µmol/l)	C_r (nmol/l)	$J_{net,u,AP}$	$J_{net,e,BL}$
Control				
3	1.77 ± 0.16^b	0.53 ± 0.08^b	4.48	0.09
10	6.20 ± 0.25^a	2.03 ± 0.38^b	11.1	0.31
100	20.6 ± 2.37^a	20.6 ± 2.5^b	32.2	1.02
DHA				
3	2.97 ± 0.41^a	0.33 ± 0.05^b	4.15	0.08
10	5.26 ± 0.48^a	1.85 ± 0.09^b	10.9	0.14
100	20.25 ± 2.8^a	19.5 ± 3.4^b	31.8	0.39
Taurine				
3	0.43 ± 0.05^d	1.47 ± 0.16^a	0.64	0.13
10	1.35 ± 0.09^c	7.50 ± 0.92^a	2.06	0.41
100	8.54 ± 0.71^b	38.85 ± 4.10^a	14.5	2.59
DHA + taurine				
3	1.09 ± 0.12^c	1.25 ± 0.15^a	1.09	0.16
10	3.47 ± 0.45^b	5.48 ± 0.44^a	3.47	0.38
100	21.8 ± 2.7^a	30.0 ± 2.71^a	21.8	4.15

Intracellular taurine concentration (C_i) was measured in cells incubated for 5 min with increasing taurine concentrations (C_0) in the apical compartment. For calculation, a mean monolayer volume of 2.14 µl was considered (Roig-Pérez et al. 2005). The receiver concentration (C_r) is the concentration of taurine attained in the basolateral compartment as a result of monolayer incubation for 5 min with C_0 present in the apical compartment. Results are expressed as mean ± SEM of $n = 3$ monolayers. Mean values bearing different superscripts are significantly different ($P < 0.05$). Net apical uptake ($J_{net,u,AP}$) and net basolateral efflux ($J_{net,e,BL}$) were calculated from Eqs. 3 and 4 (see "Results")

in addition to the reduction in nonmediated uptake and the increase in nonmediated efflux detected for both membrane domains, resulted in a reduction in net apical uptake and in an increase in net basolateral efflux, thus explaining the lowest C_i value and the highest C_r detected in experimental conditions. This effect was not as pronounced when DHA was added to the taurine-enriched medium because the fatty acid reversed apical downregulation.

Discussion

Dietary supplementation with DHA is an increasingly common practice as it has beneficial effects on health (Moghadasian 2008). However, cellular incorporation of DHA increases the susceptibility to oxidation. In this regard, in intestinal Caco-2 cells, we have demonstrated that oxygen and nitrogen reactive species, produced as a result of DHA enrichment, are involved in the disruption of epithelial barrier function and that taurine has a protective role, counteracting the effects of hydrogen peroxide (Roig-Pérez et al. 2004).

Analysis of membrane lipid composition of cells maintained in DHA-enriched conditions shows an increase in the incorporation of this fatty acid in the brush-border membrane. This increase is higher than its increase in the culture medium (16.6- vs. 4.2-fold, respectively). Similar enrichment ratios were found for eicosapentaenoic acid (EPA, C20:5n-3), in membrane phosphatidylethanolamine and phosphatidylcholine of Caco-2 cell cultures maintained in similar growth conditions (Dias and Parsons 1995). These authors also detected that EPA enrichment was at the expense of n-6 fatty acids (mainly C20:4n-6), an effect which they attributed to the inhibition of Δ^6 and Δ^5 desaturases. These enzymes are involved in the conversion of C18:2n-6 to C20:4n-6 and C18:3n-3 to C20:5n-3. The sequence of reactions involving Δ^6 desaturation, elongation and Δ^5 desaturation is also sensitive to feedback inhibition by DHA (Chen and Nilsson 1993). In our experiments, DHA enrichment resulted in a reduction of the interconversion products from C18:2n-6, which was statistically significant for C20:2n-6 and C20:3n-6 and for the chain elongation product C22:4n-6. Similarly, the increase in membrane incorporation of n-3 fatty acids in rats fed a fish oil–based diet and chickens on a linseed oil diet was accompanied by a decrease in C20:4n-6 (Ferrer et al. 2003; Kruger et al. 1995). The increased amount of C18:2n-6 that we detected in DHA-enriched membranes can thus be attributed to a lower utilization of this fatty acid as a precursor from the desaturation–elongation sequence. The effect of DHA enrichment on n-3 fatty acid incorporation was less pronounced. In Caco-2 cells, as in rat liver, Δ^6 desaturase shows a higher affinity for n-3 than for n-6 fatty acids (Chen and Nilsson 1993). In contrast, the increase in C20:4n-3 incorporation can be attributed to a significant inhibition of Δ^5 desaturase. Chen and Nilsson (1993) found a significant increase in C22:5n-3 incorporation after EPA enrichment, which was explained by the capacity of the cells to limit the accumulation of EPA (an eicosanoid precursor) by converting it to a biologically less active fatty acid, C22:5n-3.

Dietary fatty acids can modify intestinal lipid composition and, therefore, the physicochemical properties of the membrane. This, in turn, can affect both passive diffusion and the activity of the transport systems involved in nutrient absorption (Ferrer et al. 2003). However, we observed that membrane DHA enrichment did not affect apical or basolateral passive uptake or efflux. This finding is consistent with the results of Wahnon, Cogan and Mokady (1992), who showed that changes in fatty acid unsaturation are not necessarily correlated with changes in membrane fluidity since alterations in composition can be counteracted by variations in the distribution of phospholipids.

In Caco-2 cells, DHA enrichment did not affect apical taurine transport but prevented the downregulation effect exerted by taurine and increased the capacity for basolateral taurine uptake. In diverse epithelia, the activity of system TAUT is subject to adaptive regulation by taurine availability (Han et al. 2006; Kang et al. 2002; Satsu et al. 2003; Shimizu and Satsu 2000). Downregulation induced by increased intracellular taurine concentration is related to a decrease in TAUT mRNA (Bitoun and Tappaz 2000; Kang et al. 2002; Shimizu and Satsu 2000). In Caco-2 cells, the reduction in apical taurine transport has been described as being mediated by a decrease in carrier affinity and transport capacity (Shimizu and Satsu 2000). In contrast, our results show only a decrease in carrier affinity, which was observed for both apical and basolateral taurine transport. In fact, these results constitute the first evidence of basolateral taurine downregulation in intestinal cells, which has only been previously described for renal LLC-PK1 cells (Han et al. 2006).

In a previous study, we characterized transepithelial taurine transport in Caco-2 cells by analyzing kinetic apical and basolateral uptake and efflux parameters (Roig-Pérez et al. 2005). The results obtained in that study confirmed the participation of system TAUT in taurine uptake in the apical membrane, as described in Caco-2 and HT29 cells (Brandsch et al. 1993; Shimizu and Satsu 2000). We also showed that the properties of basolateral taurine uptake were similar to apical transport. The kinetic data reported here in DHA- and taurine-enriched conditions confirm all these results. Wersinger et al. (2000) detected the participation of a low-affinity transport mechanism in addition to the high-affinity TAUT system. These authors attribute the expression of a low-affinity mechanism to the fact that the cells they used were obtained from a supraclavicular metastasis of a colon carcinoma, while Caco-2 and HT29 were derived from primary colon carcinoma. Anderson, et al. (2008) also report the participation of a low-affinity transport system in Caco-2 cells, which has been identified with system PAT1.

Calculation of unidirectional and transepithelial taurine fluxes revealed that apical uptake is higher than basolateral efflux rates, thereby enabling epithelial cells to accumulate taurine. Our present results show a reduction in basolateral efflux for DHA-enriched conditions. This effect would produce an increase in intracellular taurine concentration, confirmed for the lowest substrate concentration, in parallel with a reduction in apical-to-basolateral taurine transport (C_r). Nevertheless, this variable was not affected. In a previous study we observed that Caco-2 cell incubation with DHA increased paracellular permeability, an effect which was prevented by taurine (Roig-Pérez et al. 2004). Therefore, the reduction in transcellular taurine transport in DHA-incubated cells might be accompanied by an increase in paracellular taurine influx, so the C_r values would remain unaltered. In taurine-enriched conditions, the decrease in net apical uptake was more pronounced for the highest substrate concentrations since this parameter is determined mainly by changes in carrier affinity. In contrast, basolateral efflux increased independently of substrate concentration. These changes may explain the experimental results obtained in this condition: a reduction in intracellular taurine concentration in parallel with an increase in basolateral taurine concentration. Thus, apical taurine uptake was reduced while the amino acid accumulated in the cells flowed out through the basolateral membrane.

The physiological significance of taurine downregulation has been attributed to the contribution of intestinal and renal epithelia to taurine body pool regulation (Shimizu and Satsu 2000; Tappaz 2004). Nevertheless, the presence of a regulatory mechanism in enterocytes for maintaining intracellular taurine concentration at a certain level suggests that this amino acid plays an additional specific role in the intestine. Our results showing that apical downregulation is accompanied by basolateral taurine loss would support this hypothesis. In astrocytes, TAUT downregulation contributes to cell volume recovery after exposure to hypertonic conditions (Bitoun and Tappaz 2000). Nevertheless, the cooperative functional relationship between cell shrinkage and TAUT activity has not been demonstrated in the intestine.

The effects on transepithelial taurine transport induced by the addition of DHA support the view that taurine prevents the effect of oxidative stress originated by DHA enrichment on paracellular permeability and reinforces the protective role of this amino acid. In this sense, we previously reported that incubation of Caco-2 cells with DHA increased the formation of dienes, secondary oxidation products and malondialdehyde (Roig-Pérez et al. 2004). Moreover, DHA incubation also induced the formation of hydrogen peroxide and peroxynitrite, which have been implicated in DHA-induced effects on paracellular permeability. All these effects were partially prevented by taurine, and the protective role of this amino acid was related to its capacity to counteract the effects of hydrogen peroxide. In brain endothelial cells and in Caco-2 cells, TNF-α treatment induces an increase in taurine transport, which is associated with a protective response following TNF-α-induced injury (Kang et al. 2002; Mochizuki et al. 2002). Therefore, the effect of DHA in preventing taurine downregulation in the intestine suggests that supplementation with this fatty acid triggers an increase in cellular antioxidant requirements, which cannot be satisfied because of the downregulation caused by taurine. This hypothesis is confirmed by the increase in basolateral transport capacity observed in DHA-enriched cells. Yorek et al. (1984) observed an increase in taurine transport after

DHA enrichment in retinoblastoma cells. Moreover, in retinal pigment epithelial cells, Bridges et al. (2001) detected an increase in taurine transport induced by nitric oxide production. These results support the notion that epithelial intestinal cells regulate taurine uptake and efflux to attenuate the damage produced by stress conditions and confirm the interaction of cellular redox systems with the regulation of taurine transport.

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