Omega-3 polyunsaturated fatty acids increase purine but not pyrimidine transport in L1210 leukaemia cells

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Here we show that *in vitro* supplementation of L1210 murine lymphoblastic leukaemia cells with $n-3$ polyunsaturated fatty acids results in considerable changes in the fatty acid composition of membrane phospholipids. Incubations for 48 h with 30 μM eicosapentaenoic acid (20:5, $n-3$; EPA) or docosahexaenoic acid (22:6, $n-3$; DHA) results primarily in substitution of long-chain $n-6$ fatty acids with long-chain $n-3$ fatty acids. This results in a decrease in the $n-6/n-3$ ratio from 6.9 in unsupplemented cultures to 1.2 or 1.6 for EPA and DHA supplemented cultures, respectively. Coincident with these changes in membrane fatty acid composition, we observed a 5-fold increase in the rate of adenosine (5 μM) uptake via the nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter in EPA- and DHA- supplemented L1210 cells, relative to unsupplemented cells. This seemed to result from a decrease in the K_m for adenosine from 12.5 μM in unsupplemented cultures to 5.1 μM in DHA-treated cultures. Guanosine (50 μM) transport was similarly affected by DHA with a 3.5-fold increase in the initial rate of uptake. In contrast, pyrimidine transport, as measured by uptake of thymidine and cytidine, was not similarly affected, suggesting that substrate recognition had been altered by fatty acid supplementation. Studies using [^{3}H]NBMPR showed that there was no effect of EPA or DHA on either the number of NBMPR-binding sites or the affinity of these sites for NBMPR. This observation suggests that the increases in adenosine and guanosine transport were not due to increases in the number of transporter sites but rather that EPA and DHA directly or indirectly modulate transporter function.

INTRODUCTION

Nucleosides are required nutrients for a number of tissues including haematopoietic and intestinal epithelial cells [1,2]. In addition, nucleoside analogues are frequently used in the treatment of neoplastic and viral disease. Although some nucleosides, such as adenosine, may have targets on the extracellular surface of cells [3], most nucleoside targets are intracellular. The hydrophilic nature of nucleosides necessitates their transport across the plasma membrane by specific transport proteins. Several types of nucleoside transporter have been described and include both facilitated, equilibrative, nitrobenzylthioinosine (NBMPR)-sensitive and -insensitive systems as well as multiple concentrative systems that are largely unaffected by the inhibitors of facilitated diffusional transporters (reviewed in [1,4–6]). Many cell types express multiple transporter types, including the L1210 leukaemia cell line, which possesses both facilitated and concentrative transporters [5,7]. Because transport might limit the availability of nucleosides as cellular nutrients and could also potentially determine the efficacy of nucleoside drug therapies, regulation of nucleoside transport (NT) processes has become an important field of investigation.

We have shown previously that oncogenic transformation of rat-2 fibroblasts [8] and CSF-1 treatment of bone marrow-derived macrophages [9] lead to increases in NT rates. In both systems, changes in the total rates of NT and in the relative proportions of NBMPR-sensitive and -insensitive transporter types were demonstrated. Chen et al. [10] found that *N*,*N*-dimethylformamide-induced differentiation of HL-60 cells markedly decreased the rates of uptake for several nucleosides and correlated with a similar decrease in the number of NBMPR-binding sites. Delicado et al. [11] showed that in cultured chromaffin cells, phorbol esters and secretagogues were able to inhibit NT apparently by down-regulating the number of NBMPR-sensitive transport sites. Nagy et al. [12] showed that adenosine transport in hepatocytes was inhibited by ethanol. These studies demonstrate that NT can indeed be regulated in a variety of cell types and in response to diverse stimuli.

Because nucleoside transporters are membrane-bound proteins, it is possible that changes in membrane lipid composition modulate NT activity. Modification of the fatty acid composition of plasma membranes is relatively easily achieved *in vitro*, as demonstrated in a number of tissue culture model systems [13,14]. Lipid modification of the L1210 leukaemia cell line has been shown to modulate drug (adriamycin, methotrexate and arabinosylcytosine) and amino acid uptake (α-aminoisobutyrate and taurine) [14,15] and drug toxicity of both adriamycin and arabinosylcytosine in rat-2 fibroblasts [16]. We have also recently shown that nucleoside transport across the plasma membrane of the human erythrocyte can be modulated thorugh dietary supplementation with specific fatty acids [17]. Here we show that supplementation of L1210 cultures with the $n-3$ polyunsaturated fatty acids docosahexaenoic acid (22:6, $n-3$; DHA) and eicosapentaenoic acid (20:5, $n-3$; EPA) results in increased purine transport without affecting the rates of pyrimidine transport or the affinity and number of NBMPR-binding sites.

MATERIALS AND METHODS

Cell culture

L1210 cells were purchased from ATCC and maintained in Iscove's modified Dulbecco's medium (Gibco-BRL) with 10 %

Abbreviations used: ei, equilibrative, nitrobenzylthioinosine-insensitive; es, equilibrative, nitrobenzylthioinosine-sensitive; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NBMPR, nitrobenzylthioinosine, 6-[(4-nitrobenzyl)thio]-9-D-ribofuranosylpurine; NT, nucleoside transport; PUFA, polyunsaturated fatty acid.

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(v/v) horse serum at 37 °C in an atmosphere of 5 % CO_2 in air. Exponential-phase suspension cultures were maintained at a density of 5×10^4 cells per ml in tissue culture flasks (Falcon) and passaged twice weekly. For experiments cells were plated in 175 cm^2 flasks in 100 ml of medium with vehicle alone or with 30 μM EPA (99.9 % pure, Nu-Chek Prep, Elysian, MN, U.S.A.) or 30 μM DHA (99.99 % pure, Nu-Chek Prep). Fatty acid stocks were prepared by incubating the pure fatty acids with fetal bovine serum for 1 h at 37 °C and were used immediately. Final cell densities of all cultures after 48 h lipid incubations were routinely $(0.8–1.2) \times 10^6$ cells per ml. There were no significant differences in the doubling times, maximum cell densities or cell volumes achieved with any of the lipids (doubling time 21–23 h, maximum density 3.0×10^6 cells per ml, volume 0.70 pl per cell).

Fatty acid analysis

Total lipid was extracted from approx. 5.0×10^6 cells in Na^+ buffer (3 mM K_2HPO_4, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 144 mM NaCl, 5 mM glucose, 20 mM Tris, pH 7.4) by the method of Bligh and Dyer [18]. The phospholipid fraction was separated from other lipids by TLC on silica 60 plates (Merck) in a solvent of heptane/isopropyl ether/acetic acid (60:40:3 by vol.). The origin containing phospholipids was scraped after detection with 0.1 % aminonaphtholsulphonic acid, and fatty acids were methylated after adding $C_{17:0}$ as an internal standard. Methylated fatty acids were analysed by gas-phase chromatography as described [19].

Transport assays

All steps were performed at 22 °C. Cells were washed twice with Na^+ buffer or Na^+-free choline buffer (3 mM K_2HPO_4, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 140 mM choline-Cl, 20 mM Tris, pH 7.4) and then incubated in the same buffer with or without 1 μM NBMPR for 15 min at a final cell density of 10^7 cells per ml. To begin transport, 100 μl of cell suspension was added to an equal volume of Na^+ or choline buffer containing ^{3}H-labelled nucleoside (10 μM adenosine, 50 μM guanosine, 20 μM thymidine or 50 μM cytidine), with or without 1 μM NBMPR layered over 125 μl oil (density 1.03) in Microfuge tubes. Transport was stopped by the addition of 200 μl of ice-cold 200 μM Dilazep [20] in Na^+ buffer, followed by microcentrifugation. Pelleted cells were lysed in 300 μl of 5 % Triton X-100, incubated overnight and analysed by liquid-scintillation counting with Ecolite scintillation cocktail (ICN, Costa Mesa, CA, U.S.A.). Under the conditions of our assays there were no statistical differences between the initial rates in Na^+ buffer and choline buffer. However, to avoid potential contributions of Na^+-dependent transport to total transport rates and to allow specific examination of the equilibrative-sensitive (es) and equilibrative-insensitive (ei) transporters, choline buffer only was used in the experiments examining guanosine and cytidine transport.

For adenosine kinetic studies of the es transporter, choline buffer was used, with or without 1 μM NBMPR and graded concentrations of adenosine (0.4–100 μM). A single time point of uptake (6 s), on the linear portion of the uptake curve was used to estimate the transport rates at each adenosine concentration. The es K_m and V_{max} values were estimated by subtracting uptake in the presence of NBMPR from the uptake in the absence of NBMPR and transforming data to generate Eadie–Hofstee plots. Values of ei were taken from Eadie–Hofstee plots of uptake in choline/NBMPR buffer. Values presented are the averages from four separate experiments (each with triplicate determinations).

NBMPR binding assay

Cell suspensions were prepared as described for transport assays. Binding reactions were initiated by the addition of 100 μl of cells to 100 μl of Na^+ buffer containing graded concentrations of [^{3}H]NBMPR (0.04–40 nM; Moravek Biochemicals, La Brea, CA, U.S.A.) in microwells. After 30 min at 22 °C, cells were separated from medium by vacuum filtration of cell suspensions through 96-well cellulose membrane filters (Millipore, Mississauga, Ontario, Canada). Supernatants were collected in a microtitre plate for determination of the 'free' NBMPR concentration. The cell bound fraction ('Bound') was estimated by punching the filters, solubilizing in 0.5 M KOH (37 °C for 1 h), and analysing for ^{3}H content by liquid-scintillation counting. Specific binding was calculated by subtracting values of non-specific binding (from wells that were incubated with excess non-isotopic NBMPR) from values for total binding (determined from wells that were incubated with [^{3}H]NBMPR only). The affinity (K_d) and number (B_{max}) of NBMPR binding sites was estimated by modified Scatchard analysis [21]. Values determined by this modified filter-binding method were essentially identical to values obtained by using solution binding of larger volumes and centrifuging through oil as described for transport assays.

Chemicals

Cell culture media were from GIBCO-BRL (Burlington, Ontario, Canada) and serum was from GIBCO-BRL or ICN (Costa Mesa, CA, U.S.A.). Tissue culture dishes were from Falcon (Becton Dickinson, Lincoln Park, NJ, U.S.A.). NBMPR and unlabelled nucleosides were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{3}H]NBMPR (30 Ci/mmol) was from Moravek Biochemicals. Other ^{3}H-labelled nucleosides ([*methyl*-^{3}H]thymidine, [2,8-^{3}H]adenosine, [5-^{3}H(N)]cytidine, [8-^{3}H]guanosine) were either from Moravek Biochemicals or NEN-Dupont of Canada, Ltd. (Mississauga, Ontario). Dilazep was a gift from F. Hoffman La-Roche and Co. (Basel, Switzerland).

Statistics

Initial transport rates were estimated from time courses of uptake over 0–15 s; each time point was measured in triplicate. Tangents to the computer-generated best-fit equations (Curvefit, Jandel Scientific Ltd.) were used to estimate the initial rates of uptake. Differences between treatment groups were assessed by Student's *t*-test (for differences between two data sets only) or by ANOVA followed by Student–Newman–Keuls post-test using the GraphPad software package for personal computers (GraphPad Software, Inc., San Diego, CA, U.S.A.). Differences were considered significant at $P < 0.05$.

RESULTS

L1210 cells were initially tested for sensitivity to graded concentrations of DHA and EPA and were found to tolerate concentrations up to 300 μM with little or no toxicity (results not shown). Previous investigators had used concentrations of the order of 30 μM DHA and thus, to allow comparisons to be made between our data and theirs, we chose this concentration for all subsequent studies. After incubation with 30 μM DHA or EPA for 48 h, actively proliferating cultures were pelleted, resuspended in Na^+ buffer and total lipids extracted. After TLC separation of phospholipids, fatty acids were methylated and subjected to gas chromatographic analysis. The results of averages of two separate

Table 1 Fatty acid composition of phospholipids from L1210 cells

L1210 cells were unsupplemented or supplemented with 30 μM DHA or EPA as described in the Materials and Methods section. After a further 48 h, cells were harvested and total lipids extracted. After TLC, phospholipids were isolated, methylated and separated by gas chromatography. Results are shown as the percentage of each fatty acid of total fatty acids, means $\pm$ S.E.M. for two experiments. Abbreviations used: MUFA, monounsaturated acid; ND, not detected.

Fatty acid	Control	DHA	EPA
14:0	2.4 $\pm$ 0.2	2.2 $\pm$ 0.2	1.9 $\pm$ 0.1
14:1	0.9 $\pm$ 0.1	0.2 $\pm$ 0.05	0.2 $\pm$ 0.1
15:0	1.9 $\pm$ 0.1	0.5 $\pm$ 0.2	2.6 $\pm$ 0.2
16:0	24.2 $\pm$ 1.4	18.1 $\pm$ 0.8	22.1 $\pm$ 0.5
16:1	5.1 $\pm$ 1.1	3.1 $\pm$ 0.3	0.5 $\pm$ 0.05
18:0	0.7 $\pm$ 0.05	0.9 $\pm$ 0.1	0.6 $\pm$ 0.05
18:1	16.7 $\pm$ 0.5	14.5 $\pm$ 1.5	22.7 $\pm$ 0.4
18:2, $n-6$	31.8 $\pm$ 0.7	19.4 $\pm$ 2.2	13.2 $\pm$ 1.0
18:3, $n-6$	2.8 $\pm$ 0.2	1.7 $\pm$ 0.1	1.0 $\pm$ 0.1
18:3, $n-3$	ND	1.2 $\pm$ 0.05	6.2 $\pm$ 0.2
18:4, $n-3$	ND	1.3 $\pm$ 0.05	1.1 $\pm$ 0.1
20:0	ND	1.8 $\pm$ 0.3	0.3 $\pm$ 0.05
20:1	ND	0.8 $\pm$ 0.1	0.3 $\pm$ 0.05
20:2, $n-6$	1.3 $\pm$ 0.05	0.1 $\pm$ 0.1	6.3 $\pm$ 0.7
20:3, $n-6$	ND	1.1 $\pm$ 0.1	0.2 $\pm$ 0.2
20:4, $n-6$	4.7 $\pm$ 0.6	3.8 $\pm$ 0.2	0.3 $\pm$ 0.05
20:3, $n-3$	ND	0.4 $\pm$ 0.05	0.2 $\pm$ 0.05
20:5, $n-3$	2.2 $\pm$ 0.1	0.5 $\pm$ 0.1	4.7 $\pm$ 0.7
22:0	ND	1.2 $\pm$ 0.4	0.3 $\pm$ 0.2
22:1	ND	0.5 $\pm$ 0.4	0.2 $\pm$ 0.05
22:4, $n-6$	0.9 $\pm$ 0.2	0.3 $\pm$ 0.1	0.3 $\pm$ 0.05
22:5, $n-6$	ND	0.2 $\pm$ 0.1	0.5 $\pm$ 0.1
22:5, $n-3$	ND	0.7 $\pm$ 0.5	3.4 $\pm$ 0.5
22:6, $n-3$	3.8 $\pm$ 0.3	12.5 $\pm$ 0.5	2.5 $\pm$ 0.1
24:0	ND	1.2 $\pm$ 0.05	1.4 $\pm$ 0.1
24:1	ND	1.1 $\pm$ 0.4	ND
Saturates	29.2 $\pm$ 0.3	25.9 $\pm$ 2.0	29.2 $\pm$ 1.4
MUFAs	22.7 $\pm$ 1.7	20.2 $\pm$ 2.8	23.9 $\pm$ 0.6
$n-6$ PUFAs	41.5 $\pm$ 1.8	26.6 $\pm$ 2.7	21.8 $\pm$ 2.2
$n-3$ PUFAs	6.0 $\pm$ 0.4	16.6 $\pm$ 1.2	18.9 $\pm$ 1.6
Others	0.6 $\pm$ 0.3	10.7 $\pm$ 3.0	7.0 $\pm$ 2.3
$n-6/n-3$ ratio	6.9 $\pm$ 0.1	1.6 $\pm$ 0.2	1.2 $\pm$ 0.1

Table 2 Adenosine transport in L1210 cells treated with DHA and EPA

Cells were prepared and transport assays performed in Na^+ buffer as described in the Materials and methods section. Initial rates of uptake were determined from time courses of uptake from 0 to 15 s. The final adenosine concentration was 5 μM. Rates are expressed as pmol/s per μl and are means $\pm$ S.E.M. for five experiments. Means in columns not sharing a superscript are significantly different from each other by the Student–Newman–Keuls post-test following ANOVA on log-transformed data, $P < 0.05$. Significant differences from control by the Student–Newman–Keuls post-test following ANOVA on log-transformed data: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Total transport	NBMPR sensitive (es)	NBMPR insensitive (ei)
Control	1.8 $\pm$ 0.2[a]	0.6 $\pm$ 0.1[a]	1.2 $\pm$ 0.3[a]
DHA	4.4 $\pm$ 1.1[b] **	3.4 $\pm$ 1.0[b] ***	1.0 $\pm$ 0.7[a]
EPA	4.9 $\pm$ 1.8[b] *	3.4 $\pm$ 1.6[b] **	1.5 $\pm$ 0.5[a]

Table 3 Thymidine transport in L1210 cells treated with DHA and EPA

Cells were prepared and transport assays performed in Na^+ buffer as described in the Materials and methods section. Initial rates of uptake were determined from time courses of uptake from 0 to 15 s. The final thymidine concentration was 10 μM. Rates are expressed as pmol/s per μl and are means $\pm$ S.E.M. for three experiments. Means in the same column were not statistically different from each other by ANOVA, with the Student–Newman–Keuls post-test, $P > 0.05$.

	Total transport	NBMPR sensitive (es)	NBMPR insensitive (ei)
Control	0.85 $\pm$ 0.30	0.73 $\pm$ 0.20	0.12 $\pm$ 0.04
DHA	0.84 $\pm$ 0.05	0.74 $\pm$ 0.05	0.09 $\pm$ 0.01
EPA	0.84 $\pm$ 0.10	0.76 $\pm$ 0.10	0.08 $\pm$ 0.01

experiments, each done in duplicate, are shown in Table 1. No obvious changes in saturated or monounsaturated fatty acids were noted in the phospholipid fraction. However, substantial changes in the polyunsaturated fatty acid profiles were noted in both DHA- and EPA-treated cultures. Decreases in linoleic acid (18:2, $n-6$), γ-linolenic acid (18:3, $n-6$), arachidonic acid (20:4, $n-6$) and docosatetraenoic acid (22:4, $n-6$) were demonstrated in both DHA- and EPA-treated cultures relative to unsupplemented cells. Enrichment of membranes with a variety of $n-3$ fatty acids was observed for both EPA- and DHA-supplemented cultures with the major $n-3$ fatty acids being EPA or DHA, depending on which one was initially supplemented. Because the lipid preparations used were more than 99.9% pure, the presence of multiple $n-3$ fatty acid species in supplemented cultures suggests that considerable metabolism of the EPA and DHA occurred once these lipids entered the cells. Despite some specific differences in some fatty acids between EPA- and DHA-supplemented cultures the net effect of either $n-3$ fatty acid was to decrease dramatically the $n-6/n-3$ ratio to approximately the same level. This similarity in overall $n-6/n-3$ ratio may explain the similar changes in nucleoside transport described below.

Having established that fatty acid supplementation substantially altered the fatty acid composition of L1210 membranes, we next addressed whether nucleoside transport was affected by the modified lipid composition. Transport of adenosine (5 μM) and thymidine (10 μM) was measured in control and polyunsaturated fatty acid (PUFA)-supplemented cultures by using rapid assay technology over periods of 0–15 s. Initial rates were determined from computer-generated curves with averages of triplicate determinations at each time point. For adenosine, total transport rates were doubled, relative to control cultures, with DHA or EPA treatment (Table 2). This increase was completely accounted for by an increase in the NBMPR-sensitive transporter component because there was no statistically significant change in the NBMPR-insensitive transport rates. The increase in the NBMPR-sensitive transport component represents a 5-fold increase in adenosine uptake. No such change was observed for thymidine transport (Table 3). Neither EPA nor DHA supplementation modulated the total rates of uptake of thymidine into L1210 cells, nor were the relative proportions of NBMPR-sensitive and -insensitive transport altered. Similar results were obtained with 5 μM thymidine (results not shown). Thus adenosine uptake but not thymidine uptake was sensitive to the membrane fatty acid changes induced by PUFA supplementation.

Several mechanisms could be responsible for the altered rates of adenosine uptake. Because transport only via the NBMPR-sensitive route was affected by $n-3$ fatty acids, it is possible that the number of transporter sites had been altered by the supplementation. We estimated the number of transporter sites by labelling cells under equilibrium conditions with [^{3}H]NBMPR. As shown in Table 4, neither EPA nor DHA significantly modulated the number of NBMPR-binding sites per cell or the affinity constant of these sites. Thus an increase in transporter

Table 4 ^{3}H-NBMPR binding parameters of L1210 cells treated with EPA and DHA

Cells were prepared and labelled with ^{3}H-NBMPR as described in the Materials and methods section; 2.0×10^5 cells were incubated with graded concentrations of NBMPR for 30 min at room temperature. From Scatchard plots the affinity constant (K_d) and the number of sites per cell (B_{max}) were estimated by linear regression for 11 concentrations of NBMPR each measured in duplicate. Results are the means ± S.E.M. for three separate experiments. Means in the same column were not statistically different from each other by ANOVA, $P > 0.05$, using the Student–Newman–Keuls post-test.

	B_{max} ($10^{-5} \times$ sites per cell)	K_d (nM)
Control	1.63 ± 0.06	0.14 ± 0.02
DHA	1.59 ± 0.18	0.17 ± 0.01
EPA	1.56 ± 0.11	0.21 ± 0.09

Table 5 Kinetics for NBMPR-sensitive adenosine transport in L1210

Cells were prepared as described in the Materials and methods section in choline buffer in the absence or presence of NBMPR. Uptake in 6 s was determined for graded concentrations of adenosine (0.4–100 μM) after 48 h in the absence or presence of 30 μM DHA. Kinetic parameters were determined as described in the Materials and methods section. Values are the means ± S.E.M. for four experiments. Column means with different superscripts were statistically different by Student's t-test, $P < 0.001$. All other column means were not significantly different, $P > 0.05$.

	NBMPR sensitive		NBMPR insensitive	
	K_m (μM)	V_{max} (pmol/s per μl)	K_m (μM)	V_{max} (pmol/s per μl)
Control	12.5 ± 0.4[a]	2.8 ± 0.3	116 ± 56	34 ± 16
DHA	5.1 ± 0.4[b]	2.2 ± 0.2	152 ± 37	54 ± 20

Table 6 Guanosine and cytidine transport in L1210

Cells were prepared as described in the Materials and methods section. After 48 h of incubation with or without 30 μM DHA, time courses of uptake over 0–60 s were determined for 50 μM guanosine or cytidine in choline buffer. Initial rate estimates were calculated from five independent experiments and are expressed as means ± S.E.M. Columns not sharing a superscript are statistically different by Student's t-test, $P = 0.0176$. All other column means were not significantly different, $P > 0.05$.

	Guanosine (pmol/s per μl)		Cytidine (pmol/s per μl)	
	es	ei	es	ei
Control	0.87 ± 0.30[a]	0.14 ± 0.07	3.06 ± 0.94	0.19 ± 0.02
DHA	3.04 ± 0.48[b]	0.28 ± 0.10	3.96 ± 0.35	0.20 ± 0.01

site number cannot explain the increased rates of adenosine transport.

The fact that the total number of es transport sites did not seem to be affected by DHA and EPA suggested that the specific activity of transporter or its affinity for substrate was somehow altered. To test this possibility we performed kinetic experiments by using DHA as the supplemented fatty acid, choline buffer and graded concentrations of adenosine. Uptake was measured at 6 s, a point in the linear portion of the uptake curve for the lowest and highest concentrations of adenosine used. As Table 5 demonstrates, the K_m for the es adenosine transporter seemed to be lower in DHA-treated cultures than in unsupplemented cultures. The differences in V_{max} values for the es transporter were not statistically significant. There were no differences between the K_m or V_{max} values for the ei transporter (Table 5).

To examine in more detail the substrate specificity of the fatty acid-induced changes in the activity of the es transporter, we examined the uptake of two additional nucleosides. Guanosine represented an additional purine and cytidine an additional pyridimidine. As Table 6 shows, DHA supplementation of L1210 cultures resulted in an increased uptake of guanosine (approx. 3–4-fold) but no apparent increase in cytidine transport rates. Once again the change seems to be specific to the es transporter with no significant changes in the ei transport rates for either guanosine or cytidine. The effect of the long-chain $n-3$ PUFA appears to be selective for purine nucleosides.

DISCUSSION

Transformation, ethanol, growth factor stimulation and chemically induced differentiation have all been shown to be regulators of NT function. In many of the systems so far studied, the NBMPR-sensitive route of transport seems to be the frequent target of this regulation [8–12]. The most common mechanism involves modulating the expression level of the equilibrative NBMPR-sensitive transporter. However, in the rat-2 fibroblast system an alternative mechanism must be proposed because no differences were seen in the number of NBMPR binding sites despite large differences in adenosine and thymidine transport rates in transformed compared with normal cells [8]. In ethanol-treated hepatocytes, acute ethanol treatment apparently modulates NBMPR-sensitive transport through a mechanism independent of transporter site number, whereas chronic ethanol indeed down-regulates transporter expression [12]. In the study we describe here, we have identified yet another system where transport rates seem to be modulated by a mechanism other than changes in the number of transporter sites.

EPA and DHA supplementation substantially alter the fatty acid profile of cellular phospholipids and decrease the $n-6/n-3$ ratio by 80% in L1210 cells. Because supplementation did not alter the number of NBMPR-binding sites, we suggest that it is interactions between the $n-3$ fatty acids and the transporter protein itself that are responsible for the increase in adenosine and guanosine transport rates when $n-6$ fatty acids are replaced by $n-3$ fatty acids in phospholipids. Because thymidine and cytidine transport were not similarly affected, we propose that the substrate binding site on the NBMPR-sensitive transporter is somehow affected by the lipid environment through changes in the tertiary or quaternary structure of the transport protein or accessory proteins. The observation that the K_m for adenosine was decreased in DHA-suppmented L1210 cells supports this conclusion.

It is certainly possible that fatty acid-induced changes in nucleoside transporter function could be indirect. For example, changes in lipid signalling cascades (i.e. inositol phospholipid pathways) could result in differential post-translational modification of the nucleoside transporter itself, or some accessory protein. In fact, Nagy et al. [22] have suggested that cAMP-dependent protein kinase is responsible for the ethanol-mediated changes in NBMPR-sensitive nucleoside transport in S49 lymphoma cells. Because of the unavailability of molecular probes it is not yet possible to test the possibility that any of the nucleoside transporters are regulated by reversible phosphorylation or any other post-translational modification apart from glycosylation [23].

It is unlikely that changes in adenosine metabolism downstream of the transport step are responsible for the increased uptake we have observed. Had this been so, we would have expected that all transport components would be sensitive to fatty acid composition changes. Specifically, we saw no differences between treatments for NBMPR-insensitive adenosine uptake. An increased affinity of the transport site for its substrates adenosine and guanosine seems the most likely explanation for the phenomenon we have described in this report.

L1210 is the second cell line in which we have demonstrated that fatty acid supplementation can alter nucleoside transport rates. Previously we showed [17] that erythrocytes, prepared from men fed on diets supplemented with fish oil or vegetable oil for 6 weeks, had decreased rates of thymidine, uridine and adenosine transport. The human erythrocyte expresses only the NBMPR-sensitive transporter type so that all of the effect can be explained by changes in NBMPR-sensitive transport. Because transport increases in L1210 and decreases in erythrocytes in response to *n*−3 supplementation, it is clear that the response to fatty acid supplementation could well depend both on the tissue, species and route of administration.

For L1210 cells we have shown that *n*−3 supplementation not only leads to increased nucleoside transport but also increases the cells' sensitivity to nucleoside drugs and to adriamycin [15]. In contrast, supplementation of mouse macrophages leads to altered fatty acid composition but no increase in the sensitivity to nucleoside drugs is observed. In another model we have shown that the toxicities of arabinosylcytosine and adriamycin are decreased in normal fibroblasts relative to transformed fibroblasts in response to DHA supplementation [16]. This suggests that normal and tumour cells are not equally sensitive to the effects of fatty acid supplementation. We are currently testing whether the altered sensitivity to drugs results from altered nucleoside transporter activity in the rat fibroblast model.

In addition to ethanol, cytokines and chemical inducers of differentiation, we can now add the long-chain *n*−3 polyunsaturated fatty acids to the list of potent regulators of NBMPR-sensitive nucleoside transporter activity. The mechanisms responsible could include direct physical association of fatty acids with components of the transporter itself or possible modulation of the transporter or accessory proteins by indirectly regulating cell signalling cascades. Both the specific mechanism and the extent to which fatty acids might modify transporter activity in other cell types need to be investigated.

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