The distribution and metabolism of arachidonate-containing phospholipids in cellular nuclei*

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The cell nucleus has been identified as a location to which several arachidonic acid-metabolizing enzymes are located in stimulated cells. However, little information exists describing the distribution of arachidonate-containing phospholipids associated with the nucleus or the control of their composition. In this study, nuclei isolated from human monocyte-like THP-1 cells were found to have a distribution of arachidonyl-phospholipids which is markedly different from that of other cellular membranes. THP-1 nuclei which contained 22% of total cellular arachidonate, showed a near equal distribution of arachidonate in 1-acyl-2-arachidonoyl-glycero-3-phosphocholine, 1-acyl-2-arachidonyl-glycero-3-phosphoethanolamine, 1-acyl-2-arachidonoyl-glycero-3-phosphoinositol and 1-alk-1-enyl-2-arachidonoyl-glycero-3-phosphoethanolamine molecular species. In contrast in non-nuclear membranes, arachidonate was located primarily in 1-alk-1-enyl-2-arachidonoyl-glycero-3-phosphoethanolamine molecular species which accounted for approximately half of the arachidonate in all non-nuclear phospholipids. Isolated nuclei were incapable of initially acylating arachidonic acid into their phospholipids in the absence of cellular cytosol. However, they were capable of efficiently remodelling existing arachidonate between phospholipid classes and subclasses. Isolated nuclei contained 25–30% of the cellular activity of CoA-independent transacylase, the key enzyme responsible for arachidonate-phospholipid remodelling. This enzyme is also critical in the control of arachidonate availability following cell stimulation. Given that the cellular distribution of arachidonate is such that nuclei are enriched in donor substrates for the CoA-independent transacylase reaction, that non-nuclear membranes are enriched in acceptor substrates and that nuclei have the enzymatic machinery to remodel arachidonate efficiently, these results suggest that CoA-independent transacylase may be responsible for the remodelling of arachidonate not only between different phospholipid species within the same organelles but also between different sub-cellular compartments.

INTRODUCTION

The cellular nucleus has recently generated much interest as the subcellular location of many enzymes involved in arachidonic acid (AA) metabolism. For example, cyclooxygenase II and 5-lipoxygenase activating protein have been localized to nuclear membranes while the 85 kDa phospholipase A_2 and 5-lipoxygenase are found in the cytosol of a number of cells and have the capacity to translocate to the nuclear envelope during cell stimulation [1–5]. These findings have raised important questions regarding the composition and control of levels of arachidonate-containing phospholipids which reside around the nucleus.

Mammalian cells generally contain as many as 20 arachidonate-containing phospholipid molecular species and arachidonate is moved through different phospholipids in a sequential fashion [6]. For example, AA as a free acid in circulation is moved into most cells and rapidly converted to arachidonoyl-CoA [7–9]. This arachidonoyl-CoA is then incorporated into 1-acyl-linked phospholipids by a CoA-dependent acyl transferase reaction [10–14]. Once AA is placed into 1-acyl-linked phospholipids such as 1-acyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (GPC), it is then transferred to 1-alkyl-2-lyso-GPC and to 1-alk-1-enyl-2-lyso-*sn*-glycero-3-phosphoethanolamine (GPE) by the enzyme CoA-independent transacylase (CoA-IT) [15–22]. This pathway of remodelling is selective for 20-carbon fatty acids and is believed to be important not only in the maintenance of homeostatic arachidonate levels within cellular phospholipid species throughout the cell, but also in the rapid redistribution of arachidonate into releasable phospholipid pools during stimulation of inflammatory cells [18,23,24]. Although the pathways of arachidonate uptake and remodelling have been studied in several cells, little is currently known about the subcellular location of the arachidonate-containing phospholipids or enzymes that remodel these phospholipids.

Previous studies in fibroblasts suggest that arachidonic acid is initially incorporated in glycerolipids around the nuclear envelope and then is moved into other cellular compartments [25,26]. Moreover, newly incorporated cellular arachidonate appears to move between cellular compartments in the same time frame as it is remodelled between phospholipid molecular species [25,27]. These observations raised the possibility that the nucleus is an important initial site of control for the incorporation and redistribution of arachidonate in phospholipids. Given the lack of information on arachidonate–phospholipid metabolism in the nucleus, the goal of the current study was to determine the distribution of arachidonate in nuclear phospholipids and the capacity of the nucleus to remodel arachidonate between phospholipid molecular species.

Abbreviations used: CoA-IT, CoA-independent transacylase; AA, arachidonic acid; GPC, glycero-3-phosphocholine; GPE, glycero-3-phosphoethanolamine; GPI, glycero-3-phosphoinositol; GPL, glycero-3-phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLA_2, phospholipase A_2; PS, phosphatidylserine; NL, neutral lipids; HSA, human serum albumin; FBS, fetal bovine serum; HBSS, Hanks Balanced Salt Solution; TLC, thin-layer chromatography; BMMC, murine bone marrow-derived mast cells; NICI-GC/MS, negative ion chemical ionization gas chromatography/mass spectrometry.

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METHODS

Materials

Phospholipid standards phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were obtained from Avanti Polar Lipids, Inc (Birmingham, AL, U.S.A.). Fatty acid standards were obtained from Cayman Chemical Co. (Ann Arbour, MI, U.S.A.). Pyridine and acetic anhydride were purchased from Alltech Associates Inc. (Deerfield, IL, U.S.A.). Phospholipase C from *Bacillus cereus*, Ficoll (type 400), essentially fatty acid-free human serum albumin (HSA), ethanolamine, fetal bovine serum (FBS), penicillin–streptomycin and common laboratory chemicals were obtained from Sigma Chemical Company. RPMI 1640 culture media and Hanks Balanced Salt Solution (HBSS) were obtained from Gibco (Grand Island, NY, U.S.A.). [5,6,8,9,12,14,15-^{3}H] Arachidonic acid was obtained from American Radiolabelled Chemicals Inc. (St. Louis, MO, U.S.A.). Uniplate silica gel G thin-layer chromatography (TLC) plates were obtained from Analtech Inc. (Newark, DE, U.S.A.). Silica gel columns were from Baker (Philipsburg, NJ, U.S.A.). Octadeuterated AA ($^2H_8$-AA) and trideuterated stearic acid ($^2H_3$-SA) were purchased from Biomol Research Laboratories (Plymouth Meeting, MA, U.S.A.). Pentafluorobenzyl bromide (20 % in acetonitrile) and di-isopropanolamine (20 % in acetonitrile) were purchased from Pierce (Rockford, IL, U.S.A.). All solvents (HPLC grade) were purchased from Fisher Scientific (Silver Spring, MD, U.S.A.).

Cells

The human monocytic leukaemia cell line THP-1 was obtained from the American Type Culture Collection, murine bone marrow-derived mast cells (BMMC) were prepared as previously described [18] and the murine liver mast cell line CFTL-15 was obtained from NFS/N mice and was a generous gift from Dr. Jacalyn Pierce (NIH). THP-1 cells were maintained in RPMI media containing penicillin (250 U/ml) and streptomycin (250 μg/ml) and supplemented with 10 % FBS. BMMC and CFTL-15 cells were maintained in WEHI-conditioned media prepared as previously described [18].

Preparation of nuclei

All solutions used during nuclear preparations were kept on ice. Cells were suspended in hypotonic buffer (10 mM Tris, 10 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1 mM PMSF, 1 μg/ml leupeptin, 3 mM $MgCl_2$, pH 7.5) at $(1–2) \times 10^7$ cells per ml and homogenized with 15 passes in a Potter-type Teflon-on-glass homogenizer. An aliquot of the whole cell homogenate was taken for lipid analysis and DNA quantitation as described below. The homogenate was subjected to centrifugation in a swinging bucket rotor at 700 ***g*** for 7 min at 4 °C. The supernatant fluid was discarded and the pellet was resuspended in hypotonic buffer and homogenized as above using 20 passes. The resulting suspension was layered over a 16–25 % discontinuous Ficoll gradient and centrifuged at 10000 ***g*** for 30 min in a swinging bucket centrifuge at 4 °C. The resulting nuclear pellet was resuspended in HBSS containing 5 mM $MgCl_2$.

Extraction and analysis of lipids

Lipids were extracted from whole cell homogenates or nuclear preparations by the method of Bligh & Dyer [28]. A fraction of the lipid extract containing $[^2H]_8$AA and $[^3H]_3$SA as internal standards was submitted to methanolic base hydrolysis by heating in methanol : water (75 : 25, v/v) containing 2M KOH at 60 °C for 30 min. The solution was diluted with 8 volumes of water, acidified with 1 volume of 6M HCl and the lipids were extracted by loading the solution onto Bakerbond octadecyl columns. The columns were washed with 2 ml of water and the fatty acids were then eluted from the column with 4 ml of methanol. Pentafluorobenzyl esters of fatty acids were prepared and the quantities of fatty acids were determined by negative ion chemical ionization gas chromatography/mass spectrometry (NICI-GC/MS) as previously described [29].

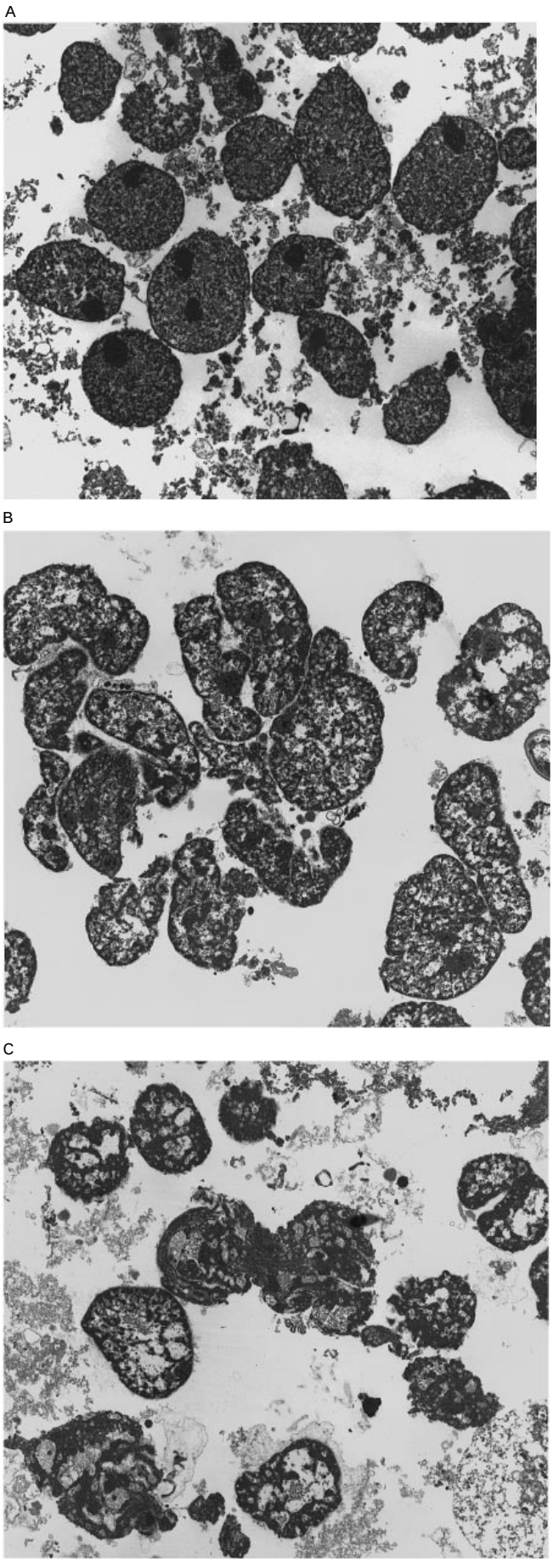
Another fraction of the extracted lipids was used to separate glycerolipid classes by HPLC using a silica column (Ultrasphere, 4.6×250 mm; Rainin Instrument Inc., Woburn, MA, U.S.A.) with a hexane : 2-propanol : ethanol : 50 mM phosphate buffer (pH 7.4) : acetic acid (490 : 367 : 100 : 30 : 0.6, by vol.) mobile phase [30]. After 5 min, the composition of phosphate buffer was increased from 3 to 5 % over a 5 min period and was maintained at 5 % for the remainder of the run. Fractions containing neutral lipids (NL), PE, PI, PS and PC were collected. The arachidonate content of each fraction was determined by NICI-GC/MS as described above.

For phospholipid subclass analysis, the PE and PC fraction was isolated by normal phase HPLC and solvents were removed under a stream of nitrogen. One millilitre of 100 mM Tris buffer (pH 7.4) was added to the dried lipids. Phospholipase C, 40 U and 20 U was added to the PE and PC fractions, respectively, followed by 2 ml of ethyl ether and the solutions were incubated for 6 h at 37 °C in a shaking water bath. The resulting diglycerides were extracted with 2 ml hexane and then again with 2 ml hexane : ether (1 : 1, v/v). Solvents were removed from extracts under a stream of nitrogen and diglycerides were acetylated by incubating overnight in pyridine : acetic anhydride (1 : 5, v/v) at 37 °C in a shaking water bath. The solvents were then dried under N_2 and the lipids were extracted twice with ether : hexane (1 : 1, v/v). The extract was washed once with 1 ml water and the subclasses were separated by TLC using a benzene : hexane : ether (50 : 45 : 4, by vol.) mobile phase. The areas containing 1-acyl, 1-alkyl and 1-alk-1-enyl-linked lipids were identified by co-migration with standards which were visualized with iodine. The TLC scrapings were extracted with ether : methanol (9 : 1, v/v) and were exposed to base hydrolysis as described above. Fatty acids were then derivatized and analysed by NICI-GC/MS as described above.

Total lipid phosphorus was determined colorimetrically [31]. In some experiments, phosphorus was assayed directly on TLC scrapings following the separation of lipid classes by TLC using a chloroform : methanol : acetic acid : water (50 : 25 : 8 : 3, by vol.) mobile phase.

Labelling of nuclei with [^{3}H]arachidonic acid

In experiments where cells were labelled with [^{3}H]AA (200 Ci/mmol), cells were washed by centrifugation once with HBSS and resuspended at 20×10^6 cells per ml in Ca^{2+}-free HBSS. [^{3}H]AA (2 μCi/2×10^7 cells) was added in 200 μl of HBSS containing HSA (250 μg/ml) and the cells were incubated for 10 s at 37 °C. The cells were then washed once by centrifugation at 4 °C with ice-cold HBSS containing 250 μg HSA/ml, and were then resuspended in HBSS at 37 °C for the indicated incubation times. In parallel experiments, nuclei were prepared as described above and resuspended in 250 μl HBSS containing 5 mM $MgCl_2$ or in 200 μl HBSS with 5 μM $MgCl_2$ supplemented with 50 μl of cellular cytosol (1×10^6 cell equivalents). To this solution [^{3}H]AA (5 μCi) was then added in 50 μl of HBSS containing HSA (250 μg/ml) and the suspension was allowed to incubate for



15 min at 37 °C. The nuclei were then washed by centrifugation at 4 °C with HBSS containing 5 mM $MgCl_2$ and HSA (250 μg/ml) ($\times$ 2), were resuspended in HBSS containing 5 mM $MgCl_2$ and were incubated in the presence or absence of 25 μM CoA-IT inhibitor SK & F98625 at 37 °C. At the indicated times, aliquots of the incubations were removed, the lipids were extracted, and the phospholipid classes were separated by HPLC as described above. The radioactivity associated with each phospholipid class was measured by liquid scintillation spectroscopy. In some experiments subclasses of PE and PC species were then separated by TLC as described above, and the radioactivity associated with each subclass was measured by liquid scintillation spectroscopy.

CoA-IT activity

CoA-IT activity was measured as described previously [32]. Nuclear preparations or whole cell homogenates (5–20 μg protein) were diluted in PBS with 1 mM EGTA to the desired protein concentration. Reactions were initiated by the addition of [1-^{3}H]alkyl-2-lyso-GPC (0.1 μCi/tube) and unlabelled 1-alkyl-2-lyso-GPC (1 μM final concentration) in assay buffer (with 250 μg/ml BSA). The reaction mixture was incubated for 10 min at 37 °C. The reaction was stopped, the lipids were extracted [28] and separated by TLC in chloroform/methanol/acetic acid/water (50:25:8:2.5, v/v). The radioactive profile of products was visualized by radioscanning (BioScan), the products were scraped and were then quantified by liquid scintillation spectroscopy.

Electron microscopy

Nuclei were fixed with 2.5 % glutaraldehyde in 100 mM phosphate buffer, pH 7.3. Samples were then prepared for transmission electron microscopy and samples to be sectioned were embedded in Spurr Resin (Polysciences, Warrington, PA). Thin sections (0.1 μm) were visualized at 80 keV in a Phillips EM-400.

DNA measurement

In all experiments, the amount of an arachidonate-containing glycerolipid was normalized to DNA content. DNA was measured as previously described by fluorometry using the Hoechst 33258 dye [33].

RESULTS

Nuclear preparations

In order to investigate the distribution of arachidonate in the nucleus it was important to ensure that pure nuclear preparations were obtained. Nuclei were prepared from several myeloid cell lines, utilizing a technique in which cells were disrupted by homogenization in a hypotonic solution and nuclei were isolated by centrifugation through a density gradient. This method requires minimal sample processing, thus reducing the potential for the mixing of lipids from different cellular compartments following cell disruption. Figure 1 shows electron micrographs of nuclear preparations from three cell types, THP-1, CFTL-15 and BMMC. Nuclear preparations from both THP-1 cells and CFTL-

Figure 1 Transmission electron micrographs of nuclear preparations from THP-1 cells (top), murine CFTL-15 cells (centre) and murine bone marrow-derived mast cells (BMMC) (bottom).

Table 1 The distribution of arachidonate in lipid classes extracted from THP-1 nuclear preparations and of non-nuclear lipids

Lipids were extracted from total cell homogenates or from nuclear preparations. The lipid classes were separated by normal phase HPLC and the arachidonate content of NL, PE, PI/PS and PC was determined by NICI/GC-MS analysis as described in the Methods section. The non-nuclear values of arachidonate were obtained by measuring the arachidonate mass per μg DNA for each phospholipid class in nuclear preparations and subtracting this value from arachidonate mass per μg DNA for each phospholipid class in the total cellular extracts. DNA was measured as described in the Methods section. The data are the mean $\pm$ S.E.M. of five separate experiments.

	Arachidonate (ng/μg DNA)			
	NL	PC	PI/PS	PE
Nuclear	1.0 ± 0.4	4.3 ± 0.6	5.8 ± 1.3	12.5 ± 4.1
Non-nuclear	2.7 ± 0.6	12.0 ± 2.0	14.7 ± 2.6	51.2 ± 7.9

Table 2 The phospholipid composition of THP-1 cells and nuclei

Lipids were extracted, phospholipid classes were separated by TLC and the lipid phosphorus content of each class was measured as described in the Methods section. Values are expressed as the means $\pm$ S.E.M. ($n = 3$). There were no significant differences ($P \leqslant 0.05$) between values in different rows as determined by a two-tailed paired Student's t test analyses.

	Lipid phosphorus (%)		
	PC	PI/PS	PE
Total cell	48 ± 1.7	18 ± 2.1	34 ± 1.1
Nucleus	45 ± 3.6	25 ± 2.9	30 ± 1.8

15 routinely contained intact nuclei that were separated from most cellular debris. Nuclear preparations from BMMC cells and human neutrophils (not shown), however, yielded nuclei that were not completely dissociated from other cellular structures. THP-1 cells were therefore utilized for all further experiments. The decrease in the specific activities of marker enzymes for Golgi, endoplasmic reticulum, mitochondria and plasma membrane in nuclear preparations from THP-1 cells compared to whole cell homogenates confirmed the purity of the nuclear preparations (data not shown).

Analysis of arachidonate in nuclei from THP-1 cells

The amount of cellular arachidonate associated with nuclear preparations varied in the different cell types. When arachidonate mass was normalized to DNA content 41 ± 7, 22 ± 3 and $48 \pm 10\,\%$ of the cellular arachidonate was associated with the nucleus in BMMC, THP-1 and CFTL cells, respectively. The distribution of the nuclear arachidonate in phospholipid classes was then compared to that of the rest of the cell. Table 1 shows the mass distribution of both nuclear and non-nuclear arachidonate in phospholipid classes from THP-1 cells. The values for the non-nuclear arachidonate were obtained by measuring the arachidonate mass per μg DNA for each phospholipid class in nuclear preparations and subtracting this value from arachidonate mass per μg DNA for each phospholipid class in the total cellular extracts. While PE was the most abundant arachidonate-containing class in both nuclear and non-nuclear compartments, PE contained 1.8-fold more arachidonate than all other glycerolipid pools combined in the non-nuclear fractions, whereas in nuclear preparations, PE contained roughly the same quantity of arachidonate as all other glycerolipids combined. The difference in the distribution of arachidonate in the nucleus and non-nuclear fractions was not due to a greater content of total PE (containing all fatty acids) in the non-nuclear fractions since there were no differences in the distribution of total lipid phosphorus in nuclei and whole cells (Table 2).

While the experiments described above showed some differences in the distribution of arachidonate in phospholipid classes, it was not until these classes were further separated into subclasses that pronounced differences were observed. These experiments revealed that the enrichment of the non-nuclear compartment in arachidonate-containing PE is largely due to the abundance of 1-alk-1-enyl-2-arachidonoyl-PE (Table 3). This molecular species accounts for approx. 70 % of the arachidonate-containing PE in the non-nuclear fraction and was more than twice as abundant as any other arachidonate-containing species in the non-nuclear compartment. In contrast, 1-acyl-linked molecular species are the predominant arachidonate-containing phospholipids in the nucleus with 1-acyl-2-arachidonoyl-GPI being the most abundant arachidonate-containing species. In fact, approximately equivalent amounts of 1-acyl-2-arachidonoyl-GPC, 1-acyl-2-arachidonoyl-GPE, 1-acyl-2-arachidonoyl-GPI and 1-alk-1-enyl-2-arachidonoyl-GPE were found in the nuclear lipid fraction. Very little 1-alkyl-2-arachidonoyl-GPC, the major cellular precursor for platelet activating factor, was found in the nucleus. The predominance of 1-acyl-linked molecular species in the nucleus was further emphasized when the ratio of 1-ester- to 1-ether-linked phospholipids was compared in the nuclear and non-nuclear fractions. The nucleus contained a 1.5:1 ratio of arachidonate in 1-ester-linked species relative to 1-ether-linked species (contains both 1-alkyl and 1-alk-1-enyl species), while non-nuclear membranes had a 1-ester to 1-ether ratio of 0.6:1.

CoA-IT activity and remodelling of arachidonate in nuclear preparations

1-Acyl-linked phospholipids are proposed to be the major donors of arachidonate in arachidonate-phospholipid remodelling (orchestrated by the CoA-IT reaction) and 1-ether-linked phospholipids are proposed to be the major acceptor phospholipids of arachidonate in this reaction. The observation that the nucleus is enriched in the major natural donor substrates of CoA-IT (1-acyl-2-arachidonoyl-linked phospholipids) in association with previous reports in the literature which show that newly incorporated AA is associated with the nucleus and is subsequently shuttled to other cellular locations [25,26], lead to the hypothesis that CoA-IT may orchestrate not only the remodelling of arachidonate between phospholipid subclasses, but also the subcellular redistribution of cellular arachidonate. Therefore, subsequent experiments were designed to determine whether nuclear preparations contained CoA-IT activity and whether arachidonate-labelled nuclei had the capacity to remodel arachidonate. Since nuclei had never been reported to exhibit CoA-IT activity, nuclear preparations were assayed for CoA-IT activity using [^{3}H]1-alkyl-2-lyso-GPC as the acceptor substrate. The nuclear preparations contained CoA-IT activity and were able to acylate the [^{3}H]1-alkyl-2-lyso-GPC substrate at a rate of 0.23 ± 0.02 μmol$\cdot\mu$g DNA$^{-1}\cdot$min^{-1} while whole cell preparations had a specific activity of 1.01 ± 0.26 μmol$\cdot\mu$g DNA$^{-1}\cdot$min^{-1}.

The capacity of the nucleus to remodel arachidonate was also assessed using two other approaches. Firstly, isolated nuclei were incubated with [^{3}H]AA in an attempt to label nuclear phospholipids and subsequently determine whether the incorporated arachidonate would be remodelled following an incubation

Table 3 The distribution of arachidonate in phospholipid subclasses of nuclear and non-nuclear fractions from THP-1 cells

The lipid classes were separated by normal phase HPLC and the PC and PE subclasses (1-acyl-, 1-alkyl- and 1-alk-1-enyl-) were further isolated by TLC as described in Methods. The arachidonate content of each subclass was determined by GC-MS analysis. The non-nuclear values of arachidonate were obtained by measuring the arachidonate mass per μg DNA for each subclass in nuclear preparations and subtracting this value from arachidonate mass per μg DNA for each phospholipid subclass in the total cellular extracts. DNA was measured as described in the Methods. The values are the mean ± S.E. of three or five separate experiments.

	Arachidonate (ng/μg DNA)						
	PC			PE			PI/PS
Fraction	1-acyl	1-alkyl	1-alk-1-enyl	1-acyl	1-alkyl	1-alk-1-enyl	1-acyl
Nuclear	3.2 ± 1.1	1.0 ± 0.1	0.2 ± 0.1	4.6 ± 2.5	2.7 ± 1.9	5.4 ± 1.3	5.8 ± 1.3
Non-nuclear	4.9 ± 1.0	3.8 ± 0.3	1.0 ± 0.6	9.4 ± 1.0	6.4 ± 1.5	35.8 ± 5.0	14.7 ± 2.6

period. However, little or no [^{3}H]AA was incorporated into nuclear phospholipids and thus no remodelling activity could be examined following the incubation of these nuclei. It is likely that the nuclei were unable to incorporate arachidonic acid into membrane phospholipids due to the lack of acyl-CoA synthase and/or acyltransferase activity in the nuclear preparations. Therefore, an attempt was made to label nuclei with [^{3}H]AA in the presence of cytosol. Addition of cytosol proved necessary for the initial incorporation of [^{3}H]AA into isolated nuclear glycerolipids. While [^{3}H]AA was incorporated into all phospholipid classes following a 15 min pulse label, nuclear PI/PS contained nearly twice as much [^{3}H]AA as nuclear PE and 1.2-fold more than PC (Figure 2A). When these pulse-labelled nuclei were incubated at 37 °C for a 4 h chase period, there was a loss of [^{3}H]arachidonate in PC species and an increase in PE species whereas the proportion of [^{3}H]arachidonate in PI/PS decreased slightly during the incubation period. Therefore, [^{3}H]arachidonate was remodelled in a fashion that is consistent with the previously described CoA-IT-driven remodelling pathway [15–22]. The presence or absence of cytosol during this chase period had no effect on the ability of isolated nuclei to remodel [^{3}H]AA (data not shown). The CoA-IT inhibitor SK & F98625 [19] blocked this redistribution of [^{3}H]arachidonate during the chase period (Figure 2A), further confirming that this was a CoA-IT-mediated effect. This rapid remodelling of [^{3}H]arachidonate was also observed when intact THP-1 cells were pulse-labelled and subsequently incubated for a 4 h chase period (Figure 2B). Like isolated nuclei, the remodelling of [^{3}H]arachidonate was very rapid, occurring within the first 2 h of incubation. Once again, as with the nuclear preparations, the presence of SK & F98625 completely inhibited this redistribution of [^{3}H]arachidonate.

Finally, the redistribution of [^{3}H]arachidonate was assessed in the subclasses of labelled nuclei to further assure that the remodelling process was characteristic of the CoA-IT-driven pathway reported in whole cells. Table 4 shows that the remodelling of [^{3}H]arachidonate in labelled nuclei is characteristic of the CoA-IT-driven pathway with the most important redistribution of [^{3}H]arachidonate being from the 1-acyl-2-arachidonoyl-PC pool to the 1-alk-1-enyl-2-arachidonoyl-PE pool. Taken together, these data indicate that isolated nuclei contain CoA-IT activity and have the capacity on their own to remodel arachidonate between nuclear phospholipids.

DISCUSSION

It is not apparent why mammalian cells have a unique set of enzymes that incorporate and remodel arachidonate, but not other more abundant fatty acids, through multiple phospholipid pools. Since recent studies have revealed the nucleus as a key subcellular site for enzymes involved in the release and metabolism of arachidonic acid [1–5,34], the current study focused on the distribution and metabolism of arachidonate-containing phospholipids at this subcellular location. The differential distribution of arachidonate-containing phospholipids among nuclear and non-nuclear membranes reported here sheds new light on a number of previous observations. Neufeld and colleagues [26] showed by quantitative electron microscope autoradiography that following a pulse-label of murine fibrosarcoma cells with [^{3}H]AA, nuclear membranes were initially enriched in radiolabelled arachidonate and that over 24 h this label was redistributed to other cellular membranes. A similar finding was later reported by Capriotti and colleagues in human fibroblasts [25]. The present finding that the nucleus is enriched in 1-acyl-linked arachidonate-containing phospholipids together with the observation that arachidonic acid taken up by mammalian cells is initially incorporated into 1-acyl-linked phospholipids by a 1-acyl-2-lyso-*sn*-glycero-phospholipid : arachidonoyl-CoA transferase(s) shown to prefer 1-acyl-linked (versus 1-ether-linked) phospholipids [13], suggests that the movement of arachidonate observed by Neufeld may be associated with the CoA-IT-driven remodelling of 1-acyl-linked phospholipids to ether-linked phospholipids. These results are thus consistent with the hypothesis that the remodelling of arachidonate may involve not only the movement of arachidonate between phospholipid molecular species but also the movement of arachidonate from the nucleus into other cellular membranes (Scheme 1).

The current study also demonstrates that the isolated nucleus contains a relatively large amount of CoA-IT activity as well as the capacity to remodel arachidonate between nuclear phospholipids. Although most of the potential arachidonate acceptor phospholipids for the CoA-IT reaction (1-ether linked phospholipids) appear to reside in non-nuclear membranes, nuclear preparations exhibited the capacity to remodel [^{3}H]arachidonate between phospholipid classes and subclasses which was inhibited by the CoA-IT inhibitor SK & F98625. The CoA-IT reaction requires the generation of lyso-phospholipids to act as acceptors for arachidonate and, although not proven, this reaction has been assumed to be catalysed by a calcium-independent phospholipase A_2 in resting cells [35]. Therefore, the capability of isolated nuclei to remodel [^{3}H]arachidonate also indicates that these preparations likely possess the phospholipase A_2 (PLA_2) activity required to generate lyso-phospholipid acceptors.

This differential distribution of arachidonate in nuclear and non-nuclear membranes is also consistent with the subcellular location and proposed substrate specificity of different PLA_2 enzymes. For example, the high molecular mass, cytosolic

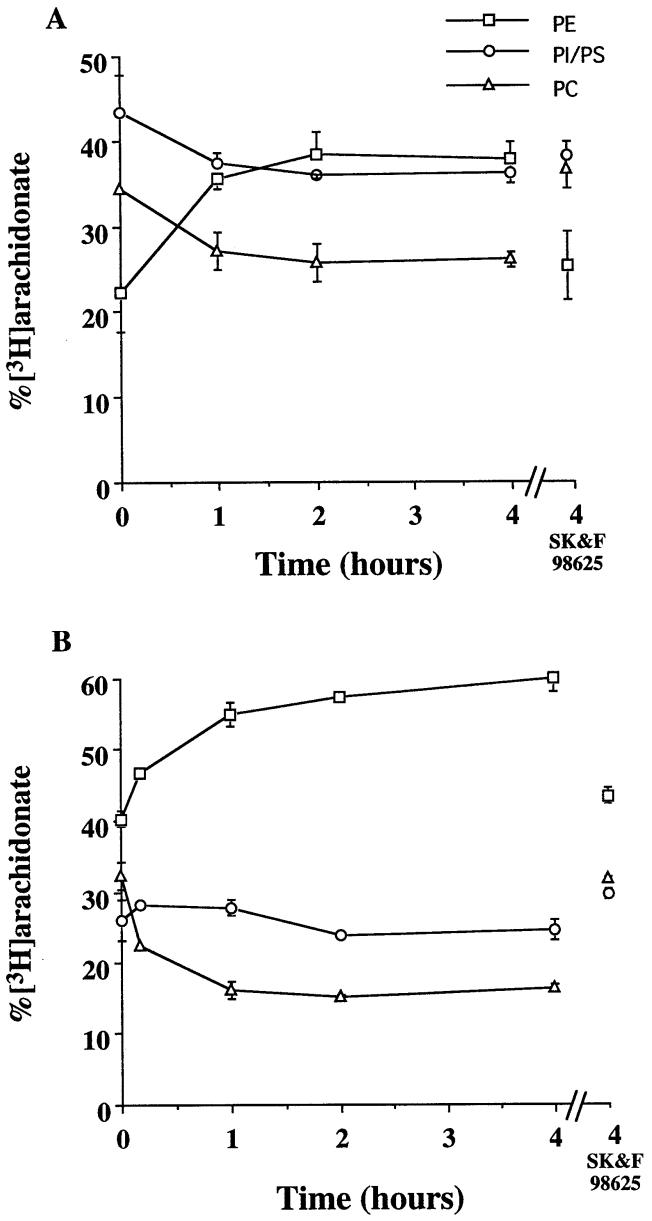


Figure 2 The distribution of [^{3}H]arachidonate in nuclear and cellular phospholipids

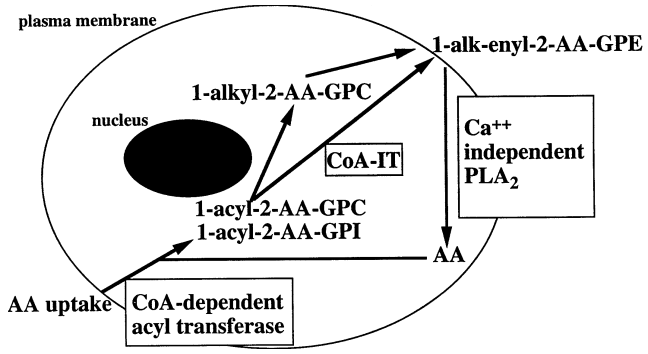
(**A**) Nuclei isolated from THP-1 cells and (**B**) THP-1 cells were pulse-labelled with [^{3}H]AA and incubated for various times at 37 °C as described in the Methods. At the indicated times, aliquots from the incubations were removed, lipids were extracted, separated by HPLC and the radioactivity associated with the different phospholipid classes was measured. The values for 4 h with SK&F98625 are from nuclei and cells that were incubated during the 4 h chase period in the presence of 25 μM of the CoA-IT inhibitor SK&F98625. Values are expressed as the means ± S.E. of four separate experiments.

Table 4 The distribution and remodelling of [^{3}H]arachidonate in cellular and nuclear phospholipid subclasses

THP-1 cells or nuclei isolated from THP-1 cells were pulse-labelled with [^{3}H]AA, washed and incubated at 37 °C as described in the Methods. At the indicated times, aliquots from the incubations were removed, lipids were extracted, phospholipid classes and subclasses were separated by HPLC and TLC, respectively, and the radioactivity associated with the different subclasses was measured. Values for whole cells are the percentage of total [^{3}H]arachidonate in cellular phospholipids associated with each subclass and are expressed as the average ± range of two separate experiments. Values for nuclei represent the percentage of total [^{3}H]arachidonate in nuclear phospholipids associated with each subclass and are expressed as the means ± S.E. of four separate experiments.

	[^{3}H]arachidonate (%)					
	Cell			Nucleus		
	1-acyl	1-alkyl	1-alk-1-enyl	1-acyl	1-alkyl	1-alk-1-enyl
PE						
$T = 0$ h	17.2 ± 2.7	4.6 ± 0.5	9.1 ± 0.5	9.5 ± 0.9	3.5 ± 0.2	6.8 ± 0.8
$T = 4$ h	14.3 ± 2.8	7.2 ± 1.0	33.4 ± 1.6	11.5 ± 0.6	4.8 ± 0.3	14.3 ± 1.4*
PC						
$T = 0$ h	21.4 ± 3.8	4.2 ± 0.7	1.2 ± 0.5	30.5 ± 1.9	4.9 ± 0.8	0.8 ± 0.1
$T = 4$ h	10.9 ± 0.3	5.1 ± 0.1	1.4 ± 0.2	17.2 ± 2.3*	4.2 ± 0.9	1.2 ± 0.3

* Denotes statistically different ($P \leqslant 0.05$) from $T = 0$ value as determined by two-tailed paired Student's t test analyses.



Scheme 1 Proposed pathway for the movement of arachidonate between nuclear and non-nuclear phospholipids

phospholipase A_2 (cPLA$_2$) is localized to the cytosol of most resting cells and then translocates to the nuclear envelope after addition of an appropriate agonist [1,2] where it is hypothesized to release arachidonate from a phospholipid pool whose specific activity in labelling experiments matches that of 1-acyl-2-arachidonoyl-GPC and GPI [36]. In contrast, low molecular mass, secretory phospholipase A_2 moves from the inside to the outside of cells during stimulation and can then hydrolyse arachidonic acid from outer membranes of the cell [37–41] from a pool that has a specific activity in labelling experiments that strongly suggests it is released from 1-alk-1-enyl-2-arachidonoyl-GPE [36]. Similar results have recently been reported in macrophages [42]. The current results strongly support the hypothesis that the different PLA$_2$ enzymes act on different AA-containing phospholipid species which reside at different subcellular locations by showing that the appropriate subcellular locations are enriched with the phospholipid substrates proposed to be hydrolysed by secretory and cytosolic phospholipases A_2. This is also consistent with results obtained in calcium ionophore-stimulated rat alveolar epithelial cells where the release of AA that is inhibited by the cPLA$_2$ inhibitor, arachidonyl trifluoromethyl ketone, is predominantly derived from nuclear phospholipids [34].

The content of arachidonate in nuclear membranes has also been speculated to have an impact on rates of cell proliferation. This concept was first postulated in liver regeneration experiments and in rat ascites hepatoma cells where the amount of arachidonate associated with the nuclei of proliferating cells is greatly diminished compared to non-proliferating cells [43].

Proliferating tumour cell lines, including THP-1 cells in the present study, have been observed to remodel arachidonate at extremely rapid rates when compared to similar non-neoplastic cells [44]. The current study suggests that this rapid remodelling could contribute to the removal of arachidonate from the nuclear membrane resulting in the elevated quantities of arachidonate in non-nuclear 1-alk-1-enyl-2-arachidonoyl-GPE [15,18]. Consistent with this hypothesis, the inhibition of CoA-IT in HL-60 cells results in the accumulation of arachidonate in 1-acyl-linked phospholipids and is associated with an inhibition of cell proliferation [45].

In conclusion, the differential distribution of arachidonate in nuclear and non-nuclear phospholipids described here provides important clues into the role of arachidonate–phospholipid remodelling in controlling arachidonate availability and in maintaining the subcellular distribution of arachidonate in resting and stimulated as well as normal and neoplastic cells. Any perturbation of this pathway resulting in an altered subcellular distribution of arachidonate may impact on the capacity of cells to mobilize arachidonate and undergo cell division.

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REFERENCES

1 Peters-Golden, M. and Mcnish, R. W. (1993) Biochem. Biophys. Res. Commun. **196**, 147–153
2 Schivella, A. R., Reiger, M. K., Smith, W. L. and Lin, L. L. (1995) J. Biol. Chem. **270**, 30749–30754
3 Brock, T. G., Paine, R. and Perters-Golden, M. (1994) J. Biol. Chem. **269**, 22059–22066
4 Woods, J. W., Evans, J. F., Ethier, D., Scott, S., Vickers, P. J., Hearn, L., Heibein, J. A., Charleson, S. and Singer, I. I. (1993) J. Exp. Med. **178**, 1935–1946
5 Morita, I., Schindler, M., Regier, M. K., Otto, J. C., Hori, T., DeWitt, D. L. and Smith, W. L. (1995) J. Biol. Chem. **270**, 10902–10908
6 MacDonald, J. I. and Sprecher, H. (1991) Biochim. Biophys. Acta **1084**, 105–121
7 Wilson, D. B., Prescott, S. M. and Majerus, P. W. (1982) J. Biol. Chem. **257**, 3510–3515
8 Laposata, M., Reich, E. L. and Majerus, P. W. (1985) J. Biol. Chem. **260**, 11016–11020
9 Bakken, A. M. and Farstad, M. (1989) Biochem. J. **261**, 71–76
10 Waku, K. and Lands, W. E. (1968) J. Lipid Res. **9**, 12–18
11 Hill, E. E. and Lands, W. E. (1968) Biochim. Biophys. Acta **152**, 645–648
12 Irvine, R. F. (1982) Biochem. J. **204**, 3–16
13 Chilton, F. H., Hadley, J. S. and Murphy, R. C. (1987) Biochim. Biophys. Acta **917**, 48–56
14 Waku, K. (1992) Biochim. Biophys. Acta **1124**, 101–111
15 Chilton, F. H. and Murphy, R. C. (1986) J. Biol. Chem. **261**, 7771–7777
16 MacDonald, J. I. and Sprecher, H. (1989) Biochim. Biophys. Acta **1004**, 151–157
17 Sugiura, T., Katayama, O., Fukui, J., Nakagawa, Y. and Waku, K. (1984) FEBS Lett. **165**, 273–276
18 Fonteh, A. N. and Chilton, F. H. (1992) J. Immunol. **148**, 1784–1791
19 Chilton, F. H., Fonteh, A. N., Sung, C. M., Hickey, D. M., Torphy, T. J., Mayer, R. J., Marshall, L. A., Heravi, J. D. and Winkler, J. D. (1995) Biochemistry **34**, 5403–5410
20 Kramer, R. M. and Deykin, D. (1983) J. Biol. Chem. **258**, 13806–13811
21 Robinson, M., Blank, M. L. and Snyder, F. (1985) J. Biol. Chem. **260**, 7889–7895
22 Masuzawa, Y, Sugiura, T., Sprecher, H. and Waku, K. (1989) Biochim. Biophys. Acta **1005**, 1–12
23 Colard, O., Breton, M. and Bereziat, G. (1986) Biochem. J. **233**, 691–695
24 Venable, M. E., Olson, S. C., Nieto, M. L. and Wykle, R. L. (1993) J. Biol. Chem. **268**, 7965–7975
25 Capriotti, A. M., Furth, E. E., Arrasmith, M. E. and Laposata, M. (1988) J. Biol. Chem. **263**, 10029–10034
26 Neufeld, E. J., Majerus, P. W., Krueger, C. M. and Saffitz, J. E. (1985) J. Cell Biol. **101**, 573–581
27 MacDonald, J. I. and Sprecher, H. (1989) J. Biol. Chem. **264**, 17718–17726
28 Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
29 Chilton, F. H. and Murphy, R. C. (1987) Biochem. Biophys. Res. Commun. **145**, 1126–1133
30 Chilton, F. H. (1990) Methods Enzymol. **187**, 157–167
31 Rouser, G., Siakotos, A. N. and Fleischer, S. (1966) Lipids **1**, 85–86
32 Winkler, J. D., Sung, C. M., Bennett, C. F. and Chilton, F. H. (1991) Biochim. Biophys. Acta **1081**, 339–346
33 Labarca, C. and Paigen, K. (1980) Anal. Biochem. **102**, 344–352
34 Peters-Golden, M., Song, K., Marshall, T. and Brook, T. (1996) Biochem. J. **318**, 797–803
35 Balsinde, J., Bianco, I. D., Ackermann, E. J., Conde-Frieboes, K. and Dennis, E. A. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 8527–8531
36 Fonteh, A. N. and Chilton, F. H. (1993) J. Immunol. **150**, 563–570
37 Fonteh, A. N., Bass, D. A., Marshall, L. A., Seeds, M., Samet, J. M. and Chilton, F. H. (1994) J. Immunol. **152**, 5438–5446
38 Pernas, P., Masliah, J., Olivier, J., Salvat, C., Rybkine, T. and Bereziat, G. (1991) Biochem. Biophys. Res. Commun. **178**, 839–844
39 Barbour, S. and Dennis, E. (1993) J. Biol. Chem. **268**, 21875–21882
40 Murakami, M., Kudo, I. and Inoue, K. (1991) FEBS Lett. **294**, 247–251
41 Murakami, M., Kudo, I. and Inoue, K. (1992) J. Biol. Chem. **268**, 839–844
42 Balsinde, J. and Dennis, E. A. (1996) J. Biol. Chem. **271**, 6758–6765
43 Ishihara, H., Tamiya-Koizumi, K., Kuriki, H., Yoshida, S. and Kojima, K. (1991) Biochim. Biophys. Acta **1084**, 53–59
44 Balsinde, J., Barbour, S., Bianco, I. and Dennis, E. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 11060–11064
45 Surette, M. E., Winkler, J. D., Fonteh, A. N. and Chilton, F. H. (1996) Biochemistry **35**, 9187–9196

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