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PHOSPHOLIPID TRANSFER PROTEIN-MEDIATED INCORPORATION AND SUBCELLULAR DISTRIBUTION OF EXOGENOUS PHOSPHATIDYLCHOLINE AND SPINGOMYELIN IN CULTURED NEUROBLASTOMA CELLS

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Murine neuroblastoma cells (strain N1E-115) were incubated with 2-[*fatty acyl*-^{14}C]acylphosphatidylcholine/sphingomyelin or phosphatidylcholine/[*choline*-^{3}H]sphingomyelin liposomes (1:1, mol/mol; 1.2 μmol total lipid/mg cell protein) in the presence of partially purified rat liver phospholipid transfer protein (2.5 mg/ml), cytochalasin B (50 μM) and 2-deoxyglucose (50 mM) for 10 min. Washed cells were chased for periods of up to 45 min at 37°C with medium containing transfer protein and unlabeled liposomes. Total transfer protein-dependent incorporation of [^{14}C]phosphatidylcholine ([^{14}C]PC) and [^{3}H]sphingomyelin was 136.7 ± 26.5(n = 5) and 23.7 ± 5.4(n = 6) nmol/mg protein per 10 min incubation, respectively, (mean ± S.D.). Incorporation of [^{14}C]PC into the mitochondrial membrane fraction was 128-fold greater (nmol/mg protein) than incorporation of [^{3}H]sphingomyelin. In contrast, incorporation of [^{3}H]sphingomyelin into a fraction enriched in plasma membrane and into microsomes was 1.4- and 2.6-fold greater, respectively, than incorporation of [^{14}C]PC. During the chase periods, the specific activities of total cellular phospholipids decreased as intact [^{14}C]PC and [^{3}H]sphingomyelin accumulated in the culture medium. In the case of cells labeled with [^{14}C]PC, the effect was due primarily to a decrease in the amount of labeled phospholipid in the mitochondrial fraction; in the case of cells labeled with [^{3}H]sphingomyelin, the decrease in activity was greatest in microsomal and plasma membrane phospholipids. The rate and extent of non-endocytotic incorporation of exogenous phosphatidylcholine into the cell membrane of cultured neuroblastoma cells, and its subsequent subcellular disposition, is different from that of exogenous sphingomyelin. Whereas PC is evidently incorporated into and turned over most rapidly in fraction enriched in mitochondrial membranes, sphingomyelin appears to be preferentially incorporated into microsomal and plasma membrane.

Introduction

Observations on a variety of cell types derived from different tissues in mammals indicate significant differences in the phospholipid compositions of the membranes comprising various subcellular organelles [1]. Plasma membrane is particularly rich in sphingomyelin; mitochondrial membranes contain a higher proportion of phosphatidylcholine (PC). The mechanism by which the specific phospholipid composition of membranes is established and maintained is to a considerable extent unknown. Biosynthesis in situ no doubt contributes to the phospholipid complement of membranes, notably microsomes; however, the transfer of preformed phospholipid and in situ revision of phospholipids previously incorporated into membranes also play a role [2,3].

Cultured neuroblastoma cell lines exhibit characteristics which make them an attractive model for studies on membrane phospholipid metabolism. The cells contain high levels of neutral, magnesium-stimulated sphingomyelinase [4,5], a neurone-specific enzyme concentrated in plasma membrane and involved in membrane phospholipid metabolism. The cells can also be induced to undergo significant differentiation-associated biochemical changes in culture [6], presumably including changes in membrane organization and metabolism.

We report here studies on the incorporation and subsequent distribution of exogenous, radiolabeled PC and sphingomyelin among various subcellular membranes of cultured neuroblastoma cells. The results show that non-endocytotic incorporation of intact exogenous PC into mitochondrial membranes and sphingomyelin into microsomal and plasma membranes occurs rapidly in the presence of rat liver phospholipid transfer protein.

Materials and Methods

Chemicals and reagents. [1-^{14}C]Oleic acid (> 98%; 57 mCi/mmol) was purchased from NEN Canada, Lachine, PQ, and [U-^{14}C]sucrose (434.6 mCi/mmol) from Amersham, Oakville, ON; 1-acyl-lysophosphatidylcholine and egg phosphatidylcholine from Serdary Research Laboratories, London, ON; bovine brain sphingomyelin, cytochalasin B and 2-deoxyglucose were from Sigma Chemical Co., St. Louis, MO, and Sephadex G-75 from Pharmacia, Montreal, PQ. All other chemicals and solvents (HPLC grade) were from Fisher Scientific, Dartmouth, NS.

2-[*oleoyl*-1-^{14}C]Oleoylphosphatidylcholine (> 97%) was prepared by esterification of 1-acyl-lysophosphatidylcholine with [1-^{14}C]oleic acid by the procedure described by Vaughan and Stanacev [7]. [^{3}H]Spingomyelin (> 98%) labeled in the choline moiety was prepared by the method described by Stoffel et al. [8]. Both compounds were purified by silicic acid column chromatography and preparative TLC [9].

Preparation of partially purified phospholipid transfer protein. Phospholipid transfer protein was partially purified by modification of the procedure described by Bloj and Zilversmit [10]. Briefly, rat liver (5–8 g) was homogenized in 5 vol. of sucrose-EDTA-Tris buffer (Buffer I: 0.25 M sucrose/1 mM EDTA/10 mM Tris; pH 7.4). After preliminary centrifugation of the homogenate at $600 \times g$ for 10 min, a crude mitochondrial pellet was isolated by centrifugation at $12\,000 \times g$ for 10 min. The supernatant was adjusted to pH 5.1 with glacial acetic acid. Precipitated protein was removed by centrifugation, and the supernatant was adjusted to pH 7.4 with solid Tris base. Proteins soluble in 50% saturated $(NH_4)_2SO_4$ and precipitated by 90% saturated $(NH_4)_2SO_4$ were dissolved in 42 mM Tris-acetate buffer, pH 7.4, containing 5 mM 2-mercaptoethanol, and were desalted by ultrafiltration. Desalted protein (60 mg) was applied to a column of Sephadex G-75 (2.6 × 90 cm) and eluted with Tris-acetate buffer at a flow rate of 60 ml/h. Phospholipid transfer protein activity in the elute was determined as described below.

Preparation of mixed phospholipid liposomes. Equimolar amounts of PC and sphingomyelin in chloroform/methanol (2 : 1, v/v) were mixed and evaporated to dryness with a stream of nitrogen. The residue was suspended in Buffer I (2 ml/μmol phospholipid) and sonicated with a Branson bath-type ultrasonic for 60 min ay 60°C. Size uniformity of the liposomes was achieved by ultrafiltration through polycarbonate membrane filters (Nucleopore, 0.2 μm) [11].

Phospholipid transfer protein assay. Aliquots (approx. 250 μg protein) of heat-treated (70°C, 20 min in Buffer I, 2 mg protein/ml) crude rat liver mitochondria were incubated with [^{14}C]PC/sphingomyelin or PC/[^{3}H]sphingomyelin liposomes (1 : 1, mol/mol; 0.2 μmol/mg protein) and phospholipid transfer protein in 42 mM Tris-acetate buffer, pH 7.4 (final volume, 1.0 ml) for 2 h at 37°C. The mitochondrial pellet was isolated by centrifugation, washed four times with ice cold Buffer I, and digested with 0.2 ml Protosol (NEN Canada) at 22°C for 60 min. After acidification, 5 ml scintillation cocktail (Beckman HP) was added and radioactivity quantitated by liquid scintillation spectrometry.

Cell culture conditions. Murine neuroblastoma cells (strain N1E-115) were cultured in Dulbecco's minimum essential medium containing 5% fetal and 5% newborn calf serum (Grand Island Biological) in 150 cm^2 Falcon flasks as described by

Palfrey et al. [12]. After 5 days, cells were harvested by gentle scraping with a rubber policeman, washed with phosphate buffered saline and finally suspended in Buffer I (approx. 6 mg protein/ml).

Cell labeling conditions. Suspensions of neuroblastoma cells in Buffer I (150 μl, approx. 900 μg protein) were preincubated in 2.1 ml Buffer I containing 50 μM cytochalasin B and 50 mM 2-deoxyglucose for 30 min at 37°C. Aliquots of [^{14}C]PC/sphingomyelin or PC/[^{3}H]sphingomyelin liposomes (1 : 1, mol/mol; $7 \cdot 10^7$ dpm; 1.2 μmol total phospholipid/mg cell protein) and phospholipid transfer protein (6 mg) were added and the volume adjusted to 6.0 ml with Buffer I. The cell-liposome-transfer protein suspension was incubated for 10 min at 37°C. The reaction was terminated by centrifugation at 8600 × *g* for 2 min at 4°C. The resulting pellet was washed twice with ice-cold Buffer I. Labeled cells (150 μg protein) were suspended in medium containing unlabeled PC/sphingomyelin liposomes (1 : 1, mol/mol; 1.2 μmol phospholipid/mg protein), transfer protein, cytochalasin B and 2-deoxyglucose and incubated for further periods of 15–45 min at 37°C. The cells were again recovered by centrifugation, washed with Buffer I, mixed with a large excess of unincubated cells (approx. 20 mg cell protein), and subjected to subcellular fractionation.

Incubations of cells (2 mg protein) for 60 min in Buffer I containing [U-^{14}C]sucrose (10^6 dpm; 435 mCi/mmol) in the presence and absence of cytochalasin B and 2-deoxyglucose showed that nonspecific, endocytotic uptake by the cells was decreased at least 50% by these inhibitors.

Subcellular fractionation. Subcellular fractionation was carried out at 4°C as described previously [5]. Cells were suspended in 1 mM sodium bicarbonate (1.3 ml/20 mg cell protein), mixed vigorously, diluted with an equal volume of 0.5 M sucrose, and centrifuged at 600 × *g* for 5 min. The pellet was suspended in 1.0 ml 0.25 M sucrose containing 0.5 mM EDTA and homogenized with 45 full strokes of the pestle in an all-glass Dounce homogenizer. The homogenate was centrifuged at 600 × *g* for 5 min, yielding a crude nuclear fraction. The supernatant was combined with the supernatant from the hypotonic bicarbonate treatment and centrifuged at 27000 × *g* for 20 min. The supernatant was further centrifuged at 100000 × *g* for 45 min to produce a microsomal pellet (P_{100K}) and cytosol. The pellet from the 27000 × *g* centrifugation was suspended in 1.0 ml 10% sucrose (w/v), layered on a discontinuous sucrose density gradient made up of 1.0 ml each of 30%, 35% and 48% (w/v), and centrifuged for 4 h at 27600 rpm in a Beckman SW 56Ti rotor, Membrane fractions were recovered with the use of a Pasteur pipet. Aliquots of each were analyzed for radioactivity, protein and/or various enzyme activities.

Protein and enzyme assays. The protein concentration of the column effluent of the Sephadex G-75 chromatographic preparation of rat liver transfer protein (see above) was determined by the method of Bradford [13]; elsewhere, protein was measured by the method described by Lowry et al. [14] with bovine serum albumin as standard. 5'-Nucleotidase was measured by the method of Avruch and Wallach [15], β-*N*-acetyl-L-glucosaminidase by the method of Okada and O'Brien [16] and carnitine palmitoyltransferase by the method of Hoppel and Tomec [17].

Thin-layer chromatography. The distribution of radiolabel among various cellular lipids after incubation of cells with [^{14}C]PC/sphingomyelin or PC/[^{3}H]sphingomyelin liposomes was determined by thin-layer chromatography [9]. Total lipids were extracted with chloroform/methanol (2 : 1, v/v) and washed [18]. Aliquots of the lower phase were spotted on layers of silica gel H (Merck), and the plates were developed with chloroform/methanol/acetic acid/water (100 : 45 : 20 : 5, by vol.). The lipids were visualized by brief exposure to I_2 and identified by reference to standards. Lipid bands were scraped into counting vials and the radioactivity measured by liquid scintillation spectrometry.

Results

The elution of rat liver phospholipid transfer protein activity from Sephadex G-75 is shown in Fig. 1. Transfer protein activity in Fractions 22–29 (fraction I), corresponding to specific phosphatidylcholine transfer protein, was used in experiments on [^{14}C]PC uptake unless otherwise indicated. Material in Fractions 31–37 (fraction II), containing nonspecific phospholipid transfer protein, was used in experiments involving [^{3}H]-

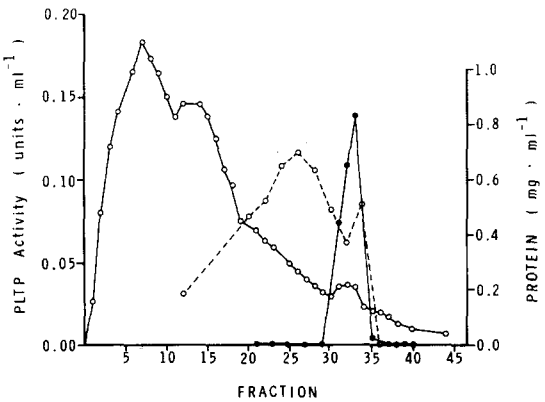

Fig. 1. Elution of rat liver phospholipid transfer protein from Sephadex G-75. Rat liver protein (60 mg) was applied to a column of Sephadex G-75 (2.6 × 90 cm) equilibrated and eluted with 42 mM Tris-acetate buffer, pH 7.4, containing 5 mM 2-mercaptoethanol at a flow rate of 60 ml/h. Fractions (5 ml) of the elutate were analyzed for protein (○———○) by the method of Bradford [11], and for phosphatidylcholine exchange activity (○------○) and sphingomyelin transfer activity (●———●) as described in the text (see Phospholipid transfer protein assay); 1 unit of activity is the number of nmoles of phospholipid transferred from liposomes to heat-treated crude mitochondria per mg of mitochondrial protein per 60 min. PLTP activity, phospholipid transfer protein activity.

sphingomyelin uptake. The incorporation of [^{14}C]PC and [^{3}H]sphingomyelin into neuroblastoma cells during 10 min incubation in the presence of cytochalasin B, 2-deoxyglucose and fraction I or II was 136.7 ± 26.5 (n = 5) and 23.7 ± 5.4 (n = 6) nmol/mg cell protein, respectively (mean ± S.D.). Incorporation using inactivated transfer protein under identical conditions was 1–7 nmol/mg protein.

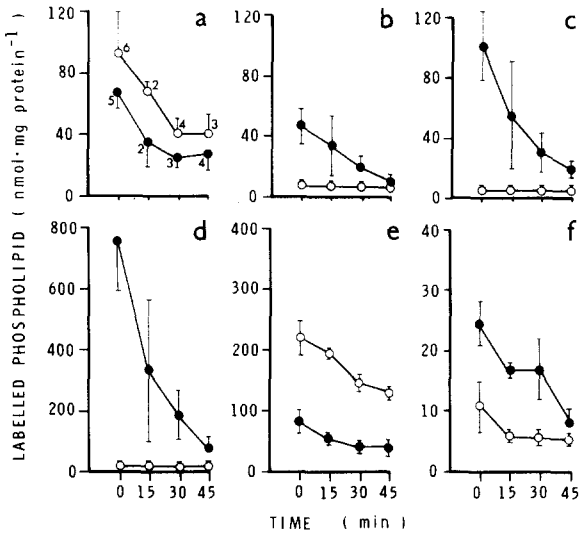

Fig. 2. Subcellular distribution of membrane associated ^{14}C and ^{3}H after incubation of intact neuroblastoma cells with [^{14}C]PC or [^{3}H]sphingomyelin. Neuroblastoma cells were harvested, pre-incubated with cytochalasin B (50 μM) and 2-deoxyglucose (50 mM) for 30 min, then incubated with [^{14}C]PC/sphingomyelin or PC/[^{3}H]sphingomyelin liposomes (1 : 1, mol/mol) in the presence of phopholipid transfer protein fractions I or II, respectively, for 10 min at 37°C. Cells were washed and transferred to media containing unlabeled liposomes, cytochalasin B, 2-deoxyglucose and transfer protein and incubated for a further 15–45 min at 37°C (see Cell labeling conditions). The cells were washed, homogenized, and subjected to differential and discontinuous sucrose density gradient centrifugation as described in the text (Subcellular fractionation). Aliquots of each subcellular fraction were analyzed for ^{14}C, ^{3}H, and protein. ●———●, Membrane-associated ^{14}C; ○———○, membrane-associated ^{3}H. Panel (a), Fraction 1; (b) Fraction 2; (c) Fraction 3; (d) Fraction 4; (e) P_{100K}; (f) cytosol. The data are means ± S.E. with the numbers of experiments shown in parentheses.

TABLE I

RELATIVE SPECIFIC ACTIVITIES OF MARKER ENZYMES IN VARIOUS SUBCELLULAR MEMBRANE FRACTIONS

The relative specific activity in each case is the ratio of the specific activity of the enzyme in the membrane fraction to that in the unfractionated cell lysate. The subcellular fractionation was carried out by differential and discontinuous sucrose density gradient centrifugation as previously described [5]. Data are presented as means ± S.E.

Marker enzyme	Relative specific activity in membrane fraction					
	1	2	3	4	P_{100K}	Cytosol
5'-Nucleotidase	10.0 ± 1.4	5.2 ± 0.7	2.6 ± 0.6	1.2 ± 0.2	4.2 ± 0.5	0.2 ± 0.03
β-Hexosaminidase	4.8 ± 1.6	6.7 ± 1.6	3.1 ± 0.6	1.5 ± 0.3	1.0 ± 0.1	0.5 ± 0.05
Carnitine palmitoyl-transferase	0.3 ± 0.2	0.5 ± 0.2	1.3 ± 0.1	5.0 ± 1.6	0.9 ± 0.3	0.6 ± 0.01

TABLE II

CHARACTERIZATION OF RADIOLABELED LIPIDS IN NEUROBLASTOMA CELLS AND CULTURE MEDIUM AFTER INCUBATION (10 MIN) IN THE PRESENCE OF [^{14}C]PC/SPHINGOMYELIN OR PC/[^{3}H]SPHINGOMYELIN LIPOSOMES AND PHOSPHOLIPID TRANSFER PROTEIN, FOLLOWED BY 45 MIN CHASE IN THE PRESENCE OF UNLABELED PHOSPHOLIPID

Data are presented as mean percentages ± S.E. of *n* experiments. SM, sphingomyelin.

Conditions	Cells/medium	*n*	LysoPC	SM	PC	Solvent front
10 min, [^{14}C]PC	cells	8	0.8±0.2	1.8±0.2	87.8±0.9	7.2±1.1
+45 min chase	cells	5	0.5±0.1	1.0±0.3	87.0±2.1	7.9±2.0
	medium	3	2.8±0.9	2.7±0.8	85.9±2.0	4.4±0.8
10 min [^{3}H]SM	cells	2	1.6±1.2	88.7±7.9	2.7±2.0	0.7±0.4
+45 min chase	cells	3	0.9±0.2	92.2±1.4	1.4±0.7	0.5±0.5
	medium	1	0.9	94.6	2.0	0.3

Fig. 2 shows the distribution of radiolabeled lipid in various subcellular fractions of neuroblastoma cells after 10 min incubation with biologically active phospholipid transfer protein and labeled liposomes and up to 45 min thereafter in medium containing the same concentration of unlabeled liposomes. During the initial 10 min incubation, incorporation of [^{14}C]PC into the membranes of Fraction 4 of the sucrose density gradient was particularly marked. In contrast, incorporation of [^{3}H]sphingomyelin was maximal into P_{100K} and membranes of Fraction 1 of the sucrose density gradient. Relatively little labeled phospholipid of either type was recovered from other subcellular membrane fractions. During the ensuing chase period, the total cell-associated radiolabel in lipid decreased by 25–50 percent. The decrease was attributable primarily to decline in the label in Fraction 4 of cells initially incubated with [^{14}C]PC/sphingomyelin liposomes and in P_{100K} and Fraction 1 of cells incubated with PC/[^{3}H]sphingomyelin (Fig. 2). When Sephadex G-75 column fraction II was substituted for fraction I in studies on [^{14}C]PC uptake, the overall incorporation of lipid was decreased, but the subcellular distribution of labeled PC was the same. In experiments in which cells were incubated with labeled liposomes in the presence of inactivated transfer protein, the small amount of [^{14}C]PC and [^{3}H]sphingomyelin incorporated into cellular membranes was evenly distributed among various subcellular membrane fractions. These was no preferential localization in any fractions, nor was there any significant difference between the distributions of [^{14}C]PC and [^{3}H]sphingomyelin.

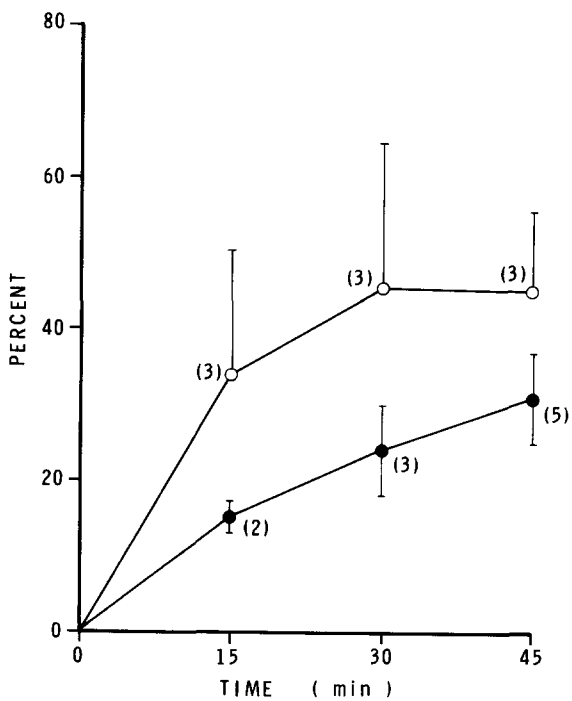

Fig. 3. Radioactivity appearing in medium during chase periods following incubation of neuroblastoma cells with [^{14}C]-PC/sphingomyelin or PC/[^{3}H]sphingomyelin liposomes. The experimental conditions were as described for Fig. 2. ●——●, ^{14}C; ○——○, ^{3}H. Data are means ± S.E. with the numbers of experiments in parentheses.

On the basis of previous studies [5] and the relative specific activities of 5'-nucleotidase, β-hexosaminidase and carnitine palmitoyltransferase shown in Table I, Fraction 1 of the sucrose density gradient, as well as the microsomal pellet (P_{100K}), was considered to be enriched in plasma membrane; Fraction 2, lysosomal membranes; and Fraction 4, mitochondrial membranes.

As cell-associated [^{14}C]PC and [^{3}H]sphingomyelin decreased during the chase period, labeled PC and sphingomyelin appeared in the culture media (Fig. 3). The distribution of the label among various lipids after 45 min incubation in the presence of unlabeled PC/sphingomyelin liposomes is shown in Table II. The results show that 90–95% of the radiolabeled lipid in the cells and the medium after initial incubation with PC/[^{3}H]sphingomyelin was still [^{3}H]sphingomyelin. In similar incubations with [^{14}C]PC/sphingomyelin, 87% of the radiolabeled lipid in the cells and 86% of that in the medium was [^{14}C]PC. The bulk of the remainder (4–8%) migrated at the solvent front in some lipid that was not characterized further.

Discussion

The results of this study show that murine neuroblastoma cells in culture take up intact PC and sphingomyelin rapidly in the presence of rat liver phospholipid transfer protein. Incorporation of labeled phospholipid into cellular membranes in the absence of active transfer protein was negligible, indicating that uptake by nonspecific adsorption, fusion or endocytosis was apparently insignificant under the conditions of the incubations. The extent to which transfer protein-mediated phospholipid exchanges alter the overall lipid compositons of membranes under various conditions is still not clear [19–23]. However, exogenous phospholipids incorporated into membranes by this mechanism are generally considered to be representative of intrinsic phospholipid rather than extraneous or adventitious.

Exogenous labeled PC and sphingomyelin incorporated into the cells rapidly became distributed among various subcellular membranes. This in itself is not surprising; LeKim et al. [24] showed that intravenously injected PC was taken up rapidly by liver and appeared intact in various subcellular membranes. We were impressed, however, by the marked difference between the subcellular distribution of [^{14}C]PC and that of [^{3}H]sphingomyelin. [^{3}H]Sphingomyelin localized primarily in P_{100K} and Fraction 1 of the sucrose density gradient; [^{14}C]PC localized predominantly in Fraction 4 and, to a lesser extent, Fraction 3 of the sucrose density gradient. In previous studies [5], we showed that Fraction 1 was enriched in plasma membrane, P_{100K} was enriched in both plasma membrane and endoplasmic reticulum, Fraction 4 was enriched in mitochondria, and Fraction 2 was enriched in lysosomal membranes.

The results suggest that an intracellular mechanism exists for directing intact exogenous PC and sphingomyelin into specific subcellular membranes. The difference in the subcellular distributions was not due to differences in regulatory properties of the rat liver phospholipid transfer protein preparations since substitution of fraction II for fraction I in experiments in which [^{14}C]PC uptake was studied did not affect the subcellular distribution of labeled PC. Neither can the difference in subcellular distribution of the two phospholipids be attributed to catabolism and differences in the recycling of labeled breakdown products. Analyses of the labeled lipid both in the medium and the cells after 45-min chase incubations showed that over 90% of the label derived from [^{3}H]sphingomyelin was recovered as intact sphingomyelin. In the case of [^{14}C]PC, less than 5% of the lipid-associated radioactivity in the medium (and less than 8% of that in the cells) appeared in less polar compounds that were not further characterized. Thus although some metabolism of the lipids appears to have occurred, this was not sufficient to account for the large differences between the distributions of PC and sphingomyelin among the membranes studied.

The chase experiments showed that in addition to being incorporated rapidly into various subcellular membranes, intact PC and sphingomyelin are rapidly exchanged back into the culture medium. The data indicate that the PC in Fraction 4 is one of the more rapidly turning over the subcellular membrane phospholipid pools. The mechanism by which this phospholipid exchanges with the PC in the medium is not clear. While the [^{14}C]PC associ-

ated with Fraction 4 membranes decreased sharply, and [^{14}C]PC appearing in the medium increased at a comparable rate during the chase incubations, fractions enriched in plasma membrane (Fraction 1 and P_{100K}) never became significantly labeled. Phospholipid transfer protein-mediated uptake of exogenous phospholipid by intact cells initially at least must involve incorporation of the lipid into plasma membrane. Dilution of labeled PC with intrinsic plasma membrane PC would then be expected to cause accumulation of label in the membrane and a relatively slower rate of turnover than that observed for other subcellular membranes. In our experiments, no accumulation of labeled PC in plasma membrane-enriched membrane fractions was observed. Moreover, although there are insufficient data to calculate accurate turnover rates, the loss of [^{14}C]PC from Fraction 4 membranes roughly parallels that from Fractions 1 and P_{100K}. These observations appear to indicate that transfer protein-mediated uptake and removal of exogenous [^{14}C]PC occurs via a small but metabolically active pool of plasma membrane PC distinct from the bulk of the membrane phospholipid. The results of analyses of marker enzymes indicated that Fraction 4 was particularly enriched in mitochondrial membranes [5]. However, the possibility exists that the [^{14}C]PC-containing membranes in this fraction actually represent a special plasma membrane-derived membrane subfraction specified by its capacity to interact with transfer protein in the medium, but one in which the specific activity of 5'-nucleotidase is apparently decreased by dilution with other membrane components of the fraction. Studies are currently in progress to explore this possibility.

In summary, our studies indicate that cultured neuroblastoma cells take up intact PC and sphingomyelin from the culture medium by a phospholipid transfer protein-mediated mechanism. Moreover, a mechanism apparently exists for directing the internal movement of exogenous phospholipid, possibly related to the regulation of intracellular movement of endogenously produced phospholipids. The mechanism by which the phospholipids in intracellular membranes, such as mitochondria, exchange with phospholipids in the culture medium remains unclear, but may involve small, distinct, metabolically active pools of lipids in the plasma membrane. The capacity to incorporate exogenous phospholipids intact into various subcellular membranes provides an additional mechanism by which environmental changes might affect the growth characteristics of cells.

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References

1 McMurray, W.C. (1973) in: Form and Function of Phospholipids (Ansell, G.B., Dawson, R.M.C. and Hawthorne, J.N., eds) pp. 205–251, Elsevier, Amsterdam
2 Wirtz, K.W.A. (1974) Biochim. Biophys. Acta 344, 95–117
3 Van den Bosch, H., Van Golde, L.M.G. and Van Deenen, L.L.M. (1972) Rev. Physiol. Biochem. Pharmacol. 66, 13–145
4 Wenger, D.A., Wharton, C. and Seeds, N.W. (1979) Life Sciences 24, 679–684
5 Spence, M.W., Wakkary, J., Clarke, J.T.R. and Cook, H.W. (1982) Biochim. Biophys. Acta 719, 162–164
6 Kimhi, Y., Palfrey, C., Spector, I., Barak, Y. and Littauer, U.Z. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 462–466
7 Vaughan, D.J. and Stanacev, N.Z. (1980) Can. J. Biochem. 58, 143–146
8 Stoffel, W., LeKim, D. and Tschung, T.S. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1058–1064
9 Rao, B.G. and Spence, M.W. (1976) J. Lipid Res. 17, 506–515
10 Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619
11 Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 557, 9–23
12 Palfrey, C., Kimhi, Y., Littauer, V.Z., Reuben, R.C. and Marles, P.A. (1977) Biochem. Biophys. Res. Commun. 76, 937–942
13 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Avruch, J. and Wallach, D.F.H. (1971) Biochim. Biophys. Acta 233, 334–347
16 Okada, S. and O'Brien, J.S. (1969) Science 165, 698–700
17 Hoppel, C.L. and Tomec, R.J. (1972) J. Biol. Chem. 247, 832–841
18 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
19 Van den Besselaar, A.M.H.P., Helmkamp, G.M., Jr. and

Wirtz, K.W.A. (1975) Biochemistry 14, 1852–1858
20 Helmkamp, G.M., Jr., Wirtz, K.W.A. and Van Deenen, L.L.M. (1976) Arch. Biochem. Biophys. 174, 592–602
21 Sandra, A. and Pagano, R.E. (1979) J. Biol. Chem. 254, 2244–2249
22 Helmkamp, G.M., Jr. (1980) Biochem. Biophys. Res. Commun. 97, 1091–1096
23 Crain, R.C. and Zilversmit, D.B. (1980) Biochim. Biophys. Acta 620, 37–48
24 LeKim, D., Betzing, H. and Stoffel, W. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 949–964