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Maintenance of Epithelial Surface Membrane Lipid Polarity: A Role for Differing Phospholipid Translocation Rates

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Summary. Large differences in lipid composition of apical and basolateral membranes from epithelial cells exist. To determine the responsible mechanism(s), rat renal cortical brush border and basolateral membrane phospholipids were labeled using ^{32}P and either [^{3}H]-glycerol or [2-^{3}H] acetate for incorporation and degradation studies, respectively. Brush border and basolateral membrane fractions were isolated simultaneously from the same cortical homogenate. Different phospholipid classes were degraded at variable rates with phosphatidylcholine having the fastest decay rate. Decay rates for individual phospholipid classes were, however, similar in both brush border and basolateral membrane fractions. In phospholipid incorporation studies, again, large variations existed between individual phospholipid classes with phosphatidylcholine and phosphatidylinositol showing the most rapid rates of incorporation. Sphingomyelin and phosphatidylserine showed extremely slow incorporation rates and did not enter into the isotopic decay phase for 48 hr. In contrast to degradation studies, however, the same phospholipid class labeled the two surface membrane domains at highly variable rates. The difference in these rates, with the exception of phosphatidylinositol, were identical to the differences in phospholipid compositions between the two membranes. For example, phosphatidylcholine was incorporated into the basolateral membrane 2.5 × faster than into the brush border membrane and its relative composition was 2.5 × greater in the basolateral membrane. The opposite was true for sphingomyelin. These results indicate incorporation and not degradation rates of individual phospholipids play a major role in regulating the differing phospholipid composition of brush border and basolateral membranes.

Key Words brush border membrane · basolateral membrane · phospholipids · epithelial polarity

Introduction

Large differences in the phospholipid composition of apical and basolateral (BLM) membrane fractions of epithelial cells such as renal proximal tubule [8, 17], small intestine [4], hepatocytes [15], and MDCK cells [23] have recently been described. The differences in renal proximal tubule cells are quite pronounced with the apical or brush border (BBM) having a sphingomyelin to phosphatidylcholine ratio of 2.1 while the BLM ratio is only 0.4 [17].

While it is generally accepted that intracellular organelles vary widely in phospholipid composition, the large differences reported for the two major surface membrane domains of the epithelial cell surface membrane presents an unique opportunity to evaluate factors important in regulating membrane lipid composition. While numerous possibilities exist to explain these differences, alterations in the rate of synthesis, translocation or degradation of phospholipids would be likely. For most cellular organelles the rate of appearance of newly synthesized phospholipids is not only dependent upon the rate of synthesis of these phospholipids at their site of origin, but also the rate of translocation from this site to the cellular organelle in question. Recent work in several laboratories has indicated that certain phospholipids, e.g. phosphatidylcholine (PC) and phosphatidylethanolamine (PE), move rapidly from their site of origin in the endoplasmic reticulum to the plasma membrane by energy and temperature-independent processes [10, 21]. Whereas other phospholipids such as sphingomyelin may originate in the Golgi [11, 13] and then move to specific subcellular organelles. Thus, cells face complex synthesis, sorting and targeting problems for phospholipids analogous to those encountered for membrane proteins. The present studies, therefore, were undertaken to determine the mechanism(s) responsible for the large differences in renal cortical BBM and BLM phospholipids.

Materials and Methods

Preparation and Characterization of Membranes

Male Sprague-Dawley rats (180–220 g) maintained on standard chow were used in all experiments, and animals were fasted prior to the i.p. injection of radioactive compounds.

Table 1. Degradation rates ($t_{\frac{1}{2}}$) of brush border (BBM) and basolateral membrane (BLM) phospholipids determined using 2-^{3}H acetate and uniformly labeled ^{3}H-glycerol[a]

	2-^{3}H acetate		^{3}H-glycerol	
	BBM	BLM	BBM	BLM
Pdt · choline	34.3	36.7	23.8	25.2
Pdt · ethanolamine	55.7	68.6	49.1	60.3
Pdt · inositol	101.9	49.9	39.2	61.9
Pdt · serine	41.8	54.1	69.4	66.6
Sphingomyelin	54.6	49.5	—	—

[a] One rat for each time point (5 points) injected with 1 mCi of either isotope i.p. 12–96 hr prior to membrane isolation. BBM and basolateral membranes were simultaneously isolated from the same cortical homogenate as described under "Materials and Methods." The $t_{\frac{1}{2}}$ was calculated over the time period when the phospholipid was in the isotope decay phase. Pdt = Phosphatidyl

Renal cortical BBM and BLM were isolated simultaneously from the same cortical homogenate and characterized enzymatically as described in detail elsewhere [16, 17]. Briefly, the procedure entailed rapid decapsulization and removal of thin cortical slices in chilled buffer (300 mM mannitol, 5 mM ethyleneglycolbis (*b*-aminoethylether)-N, N'tetraacetic acid, 18 mmol Tris (hydroxymethyl) aminomethane hydrochloride, 0.1 mM phenylmethylsulfonyl fluoride at pH 7.4). The slices from two kidneys were homogenized using a Polytron PT 200D (Brinkman) in 15 ml of buffer, and Mg^{2+} precipitation (15 mmol/liter) was carried out for 20 min. The resulting solution was centrifuged for 15 min at 2,445 × *g*, the pellet (P_2) was saved for BLM isolation, while the supernatant was centrifuged at 48,000 × *g* for 30 min to obtain crude apical membranes. This pellet was resuspended using a Potter-Elvehjem in 30 ml of diluted buffer (1 : 1 with deionized water) and taken through the Mg^{2+} precipitation process again. Enrichment for various enzymes in the apical preparation were as follows: alkaline phosphatase 12.4 ± 3.6 Na/K ATPase 1.8 ± 0.6, succinic dehydrogenase 0.5 ± 0.1, N-acetyl glucosaminidase 0.2 ± 0.1, KCN-resistant NADH-dehydrogenase 0.4 ± 0.1, and galactosyltransferase 0.6 ± 0.1.

To isolate the BLM fraction P_2 was resuspended using a loose Dounce glass homogenizer followed by repeat addition of Mg^{2+} (15 mM) and then centrifugation at 2,445 × *g* for 15 min. The pellet (P_3) was resuspended in standard buffer, diluted with water (1 : 1), and centrifuged for 15 min at 755 × *g*. The supernatant was centrifuged for 30 min at 48,000 × *g*, the resulting pellet (P_5) was resuspended in 19 ml of 50% sucrose overlayed with a discontinuous sucrose gradient using 41% (5 ml) and 38% (12 ml) in cellulose-acetate tubes and centrifuged at 88,000 × *g* at 4°C in a Beckman Model L8-70 Ultracentrifuge for 3 hr. The top layer (38%) was harvested and enzyme enrichments were Na/K ATPase 9.0 ± 1.7, alkaline phosphatase 1.7 ± 0.4, succinic dehydrogenase 1.7 ± 0.3, KCN-resistant NADH-dehydrogenase 2.2 ± 0.3 and N-acetylglucosaminidase 0.6 ± 0.3. These values are in close agreement with our previous results [16, 17].

Enzyme and Protein Measurements

Protein was measured according to Lowry et al. [14] using bovine serum albumin as a standard. Enzyme determinations were carried out using standard kinetic assays as previously reported from our laboratory [16, 17] and galactosyltransferase was assayed according to the method of Rad et al. [19].

Lipid Determinations

Lipids from approximately 1 mg of membrane protein were extracted in 6 ml of chloroform/methanol (1 : 2 vol/vol) isolated and quantitated as we have previously described [16, 17]. Total phospholipids were determined on an extract, according to Ames and Dubin [1]. Individual phospholipid species were separated by two-dimensional thin-layer chromatography on Kesilgel silica gel 60 plates, using the technique of Esko et al. [5]. Individual phospholipid species were identified using brief exposure to iodine vapor and scraped off the plates. A Bligh and Dyer [2] extraction was then carried out [6] on each individual spot, p_i was determined by the method of Ames and Dubin [1] on an aliquot, and the remainder was used to quantitate the amount of radioactivity (dpm) present.

Phospholipid Labeling

Radioisotopes were administered i.p., following an overnight fast, while the animals were under either light ether or methoxyflurane anesthesia. To evaluate for potential post-homogenization artifacts in ^{32}P-labeled phospholipids, four rats were labeled. (1 mCi ^{32}P) i.p. for either 1 or 12 hr prior to renal cortical homogenization. The homogenate was left on ice and another aliquot was collected and extracted in a similar fashion 8 hr later. Total phospholipids and individual phospholipids were separated and analyzed as described above. Following labeling for 1 hr and storage for 8 hr on ice, there was no change in the ratio of specific activities (delayed extraction/immediate extraction) for total phospholipids (0.98 ± 0.13), PC (0.97 ± 0.17), *PE* (1.06 ± 0.11) or PI (0.90 ± 0.12). Because of slow ^{32}P incorporation into sphingomyelin and PS a 12-hr labeling period had to be used. Again, there was no change in the ratio for total phospholipids (0.99 ± 0.03), PC (0.96 ± 0.16), PE (0.96 ± 0.14), PI (1.07 ± 0.12) or for SPH (1.07 ± 0.04) and PS (1.04 ± 0.28). These data indicate the level of ^{32}P-labeled phospholipids did not change following homogenization and are in agreement with similar studies in hepatocytes [20].

Miscellaneous Procedures and Materials

Radioactivity was determined using a liquid scintillation mixture (PCS, Amersham, Arlington Heights, IL) in a Packard Tri-carb liquid scintillation counter. All biochemicals were purchased from standard suppliers and were of the highest purity grades. Radiochemicals, $^{32}P_{PO_4}$ (orthophosphate), [2-^{3}H] acetate, (sp act 4.7 Ci/mmol) and uniformly labeled ^{3}H-glycerol (sp act 38.2 Ci/mmol) were purchased from New England Nuclear. All data are reported as mean ± one SD unless otherwise noted.

Results

To determine the possible role differing degradation rates play in regulating membrane phospholipid composition, both 2-^{3}H acetate and uniformly la-

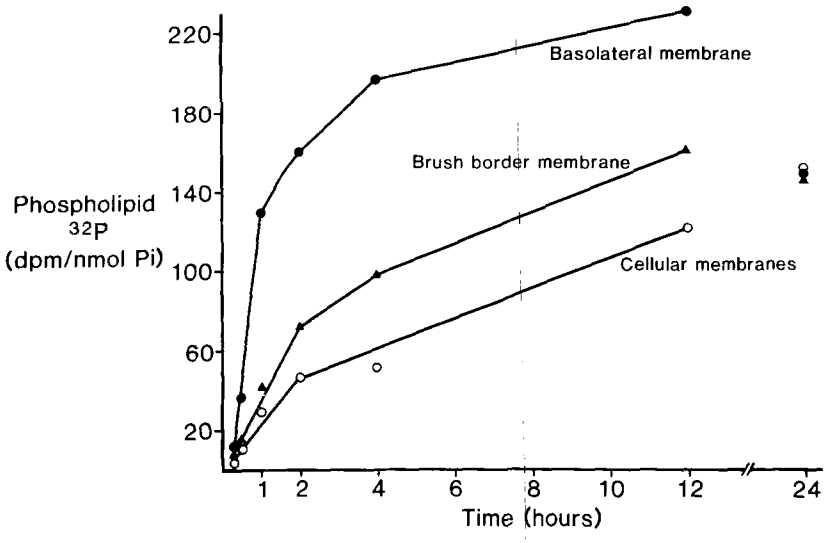

Fig. 1. Labeling of cellular, brush border and basolateral membrane phospholipids using ^{32}P. One rat for each time point was given 1 mCi ^{32}P in 1 ml normal saline i.p. and cortical BBM, BLM and homogenates were obtained at the indicated times as described under "Materials and Methods." Specific activity (dpm/nmol Pi) was calculated for cellular (○—○), brush border (▲—▲) and basolateral membranes (●—●). Cellular membranes refer to the labeling of total homogenate phospholipids

beled ^{3}H-glycerol were used. ^{32}P was not used in these studies, as its potential for reutilization is too high. The data in Table 1 indicate that after either acetate or glycerol labeling individual phospholipids are degraded at different rates. Phosphatidylcholine has the most rapid degradation rate with $t\frac{1}{2}$ of 35 and 24 hr using acetate and glycerol, respectively. The $t\frac{1}{2}$ for phosphatidylethanolamine, phosphatidylserine and sphingomyelin were all in a similar range (50–60 hr). Moreover, the degradation of these phospholipid classes, with one exception, were similar in both BBM and BLM fractions. The degradation rate for phosphatidylinositol, however, varied widely between membranes and was dependent upon whether glycerol or acetate was used as the isotopic label.

Labeling of Membrane Phospholipids

To study the rate of phospholipid appearance in BBM and BLM, ^{32}P was used as a label. ^{32}P is reabsorbed by proximal tubular cells by a Na^+-dependent process thereby giving rise to rapid and highly labeled intracellular pools [17]. Incorporation of ^{32}P into membrane phospholipids is shown in Fig. 1. Labeling of the basolateral membrane preceded BBM labeling, which in turn was labeled more rapidly than total cellular membranes. The initial incorporation into BLM and BBM was rapid, linear for up to 2 hr and occurred without an appreciable delay. Thereafter, incorporation was much slower and all three membrane fractions had the same specific activity 24 hr following injection.

The incorporation of ^{32}P into individual phospholipid classes is shown in Figs. 2–4. There was wide variability in the rate of appearance between the different phospholipids. Labeling of basolateral

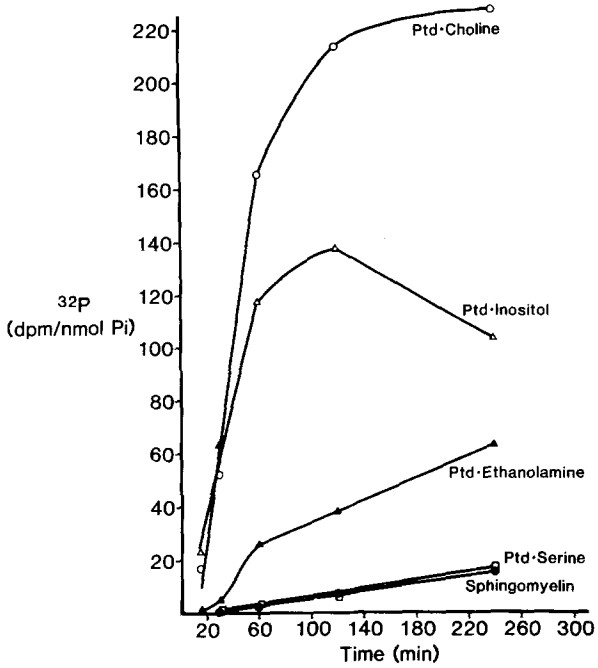

Fig. 2. Labeling of individual BLM phospholipid classes using ^{32}P. One rat for each time point was given 1 mCi ^{32}P in 1 ml normal saline i.p. and renal cortical basolateral membranes were isolated at the specified times as described under "Materials and Methods." *Pdt* = Phosphatidyl

membrane phosphatidylcholine and phosphatidylinositol was very rapid while phosphatidylethanolamine was intermediate and phosphatidylserine and sphingomyelin were labeled very slowly (Fig. 2). Brush border phospholipids showed an almost identical pattern of labeling with respect to the order of individual classes of phospholipids (Fig. 3). These experiments were repeated with similar data being obtained. For all phospholipids a linear incorpora-

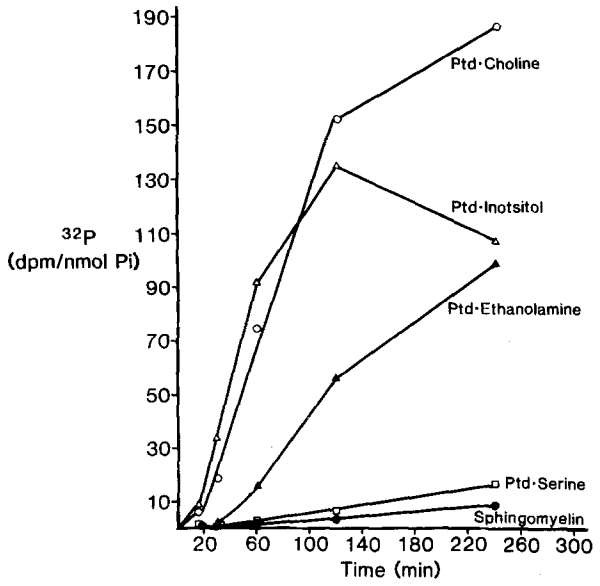

Fig. 3. Labeling of individual BBM phospholipid classes using ^{32}P. (*See* Fig. 2 for details)

tion phase could be identified and its duration depended upon the individual phospholipid class.

Large differences in phospholipid labeling for the same phospholipid class in different membranes was also noted. For example, in Fig. 4 the labeling of BLM phosphatidylcholine was much more rapid than BBM phosphatidylcholine. The reverse was noted for sphingomyelin. Labeling of both BLM and BBM sphingomyelin was extremely slow, as shown by the fact that at 24 hr the rate of incorporation of label remained linear. In fact, this phospholipid species did not enter into the isotopic decay phase until 48 hr after injection. In addition, there was a lag in its incorporation into both membrane fractions, whereas PC was incorporated immediately.

To determine the role these different incorporation rates of individual phospholipids play in determining BBM and BLM phospholipid composition, the rate of incorporation (slope) was calculated during the initial linear phase of each incorporation curve. These data are shown in Table 2. Initial rates of incorporation varied from 1.8 to 202.5 dpm/nmol Pi/min for BLM phosphatidylserine and phosphatidylcholine, respectively. Phospholipid classes also showed wide variation in incorporation rate from one membrane to the other. For example, PC was incorporated into the BLM at a rate of 250% greater than into BBM membrane. The reverse was true for sphingomyelin. When one calculates and compares the relative incorporation rates (BBM/BLM) with the relative phospholipid compositions of the two membrane fractions, the values are in very close agreement except for phosphatidylinositol.

Discussion

Recent evidence [4, 8, 15, 17, 23] clearly indicates apical and basolateral membranes of epithelial cells differ widely in lipid composition. How epithelial cells achieve and maintain this polarity is largely unknown. However, the fact that lipid polarity occurs in numerous epithelial tissues and is preserved in cultured cells [23] may indicate its importance in determining and regulating processes known to be functionally different in apical and BLM, such as ion transport [9], hormone receptors [22], electrical resistance [6] and membrane fluidity [8].

In the present studies the rate of incorporation and degradation of phospholipids in renal cortical BBM and BLM were determined. The methods used offer several advantages. First, highly enriched BBM and BLM could be isolated simultaneously from the same cortical homogenate. This eliminated variations in substrate pool size, which is desirable when using radiolabeled isotopes to measure simultaneous membrane biosynthesis. Also, to date, the isolation of the BLM from cultured cells has been reported only for virally infected cells [23]. Therefore, studies in cultured epithelial cells have been limited to evaluating the entire plasma membrane domain [20].

Several potential disadvantages of this system also exist. First, labeling studies are not strictly pulse-chase experiments and reutilization of isotopes could have occurred. This is most likely to have occurred for the isotopes used to quantitate phospholipid degradation studies. We did, however, use two chemically unrelated isotopes and were primarily concerned with the relative differences between BBM and BLM fractions. In addition, since these studies cannot yet be accomplished using cultured cells, pulse-chase experiments are not possible. We also showed that post-homogenization exchange artifacts in synthesis and degradation using ^{32}P did not occur. This was probably due to the relatively rapid fractionation process done at 0–4°C in the presence of enzyme inhibitors (0.1 mM PMSF). This was essential to establish, as major differences were found during incorporation studies. Secondly, we used 2-^{3}H acetate as a tracer to measure degradation rates. This label will only measure the rate of nonessential fatty acid loss and, therefore, does not strictly measure phospholipid turnover. Glycerol on the other hand, used to measure phospholipid backbone turnover, does not la-

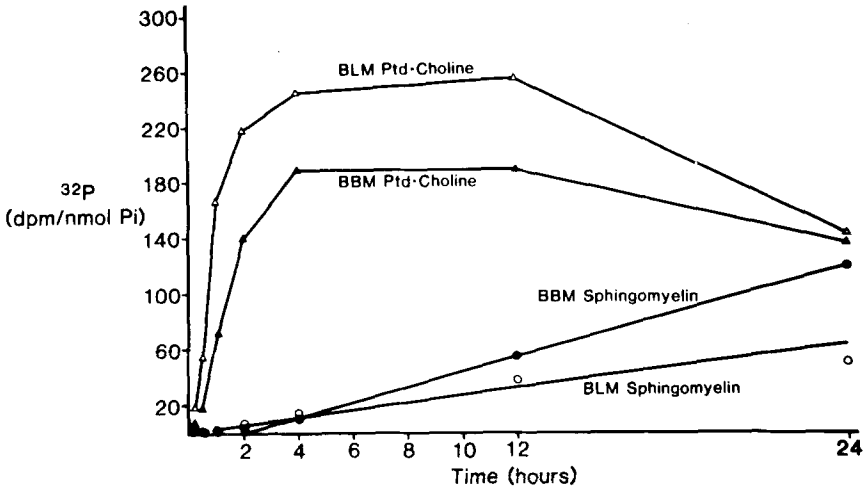

Fig. 4. Labeling of renal cortical basolateral and brush border membrane phosphatidylcholine and sphingomyelin. (*See* Fig. 2 for details.) *BBM* = brush border membrane; *BLM* = basolateral membrane

Table 2. Incorporation rate of phospholipids into basolateral and brush border membranes

Phospholipid	Incorporation rate (dpm/nmol Pi/min)		Relative values (BBM/BLM)	
	BBM	BLM	Incorporation rate	Composition
Sphingomyelin	5.4	2.4	2.5	2.6
Pdt · choline	78.9	202.5	0.4	0.4
Pdt · ethanolamine	15.9	15.0	1.1	0.9
Pdt · serine	3.4	1.8	1.9	0.4
Pdt · inositol	91.0	119.8	0.8	0.4

^{32}P (orthophosphate) was injected i.p. and brush border and basolateral membranes were simultaneously isolated at the time intervals shown in Figs. 1–3. Slopes $\left(\frac{\Delta \text{dpm/nmol Pi}}{\Delta \text{ time}}\right)$ were determined during the linear phase of uptake using at least 3 data points. All r values were greater than 0.98. Relative incorporation rates and relative compositions were calculated by dividing the brush border value by the corresponding basolateral membrane value. Pdt · = phosphatidyl.

bel sphingomyelin. Since the latter phospholipid is the major BBM phospholipid (40%), glycerol did not totally meet our objectives. The BBM and BLM fractions were not without contamination from other subcellular organelles. Ideally, one would like pure membrane fractions, but no one has been able to obtain this goal. The purity of our membrane fractions is similar to procedures used by other investigators [17]. In summary, since quantitation of incorporation and degradation rates for BBM and BLM were from the same isotopic and synthetic pool, we believe the data to be adequate for the relative comparisons in which we were primarily interested.

The results are consistent with the observation that the rate of incorporation of phospholipids and not the degradation rate is responsible for the large differences in renal BBM and BLM composition. Our work confirms that both newly synthesized PC [10] and PE [21] appear rapidly in the plasma membrane. It extends those observations to indicate that the relative rate of movement from the site of synthesis to the BBM or BLM is probably the most important factor controlling the differences in surface membrane phospholipid composition. For example, the relative composition and rate of PC movement into the BLM (Fig. 2, Table 2) was 2.5 times that of BBM. Whereas, the relative incorporation rate and composition of sphingomyelin was much higher in the BBM fraction.

An alternative explanation for the large differences in BBM and BLM sphingomyelin could be polarity of specific phospholipid synthetic enzymes to each membrane. Evidence that sphingomyelin synthesis occurs in the plasma membrane of hepatocytes is consistent with this theory [7, 24]. A relative increase in the activity of the enzyme phosphatidylcholine : ceramide phosphocholine-trans-

ferase within the BBM could therefore be responsible for its high sphingomyelin content by increasing the synthesis of sphingomyelin from PC [7, 24]. To evaluate this possibility the activity of this enzyme was measured. In preliminary studies activity was detected in the homogenate, but was not present in the BBM fraction, even in the presence of taurodeoxycholate, suggesting sphingomyelin synthesis is completed prior to movement of BBM. Other investigators have shown sphingomyelin is synthesized in the Golgi and then moves to the surface membrane [11–13]. Therefore, location of this enzyme to the plasma membrane may depend upon the cell being studied, but it does not appear to be present in renal cortical BBM.

If sphingomyelin synthesis in proximal cells does occur in the golgi apparatus, then it is important to evaluate for Golgic contamination within the BBM fraction, as it could lead to the reported increases in sphingomyelin labeling. Two lines of evidence, however, rule against this possibility. First, the lack of phosphatidylcholine : ceramide phosphocholinetransferase activity within the BBM fraction indicates there is limited Golgi contamination. Second, galactosyltransferase, a known selective enzymatic marker for the Golgi apparatus [25], was deenriched (0.6 ± 0.1) in BBM fractions. It, therefore, seems unlikely Golgi contamination leads to the large differences in surface membrane sphingomyelin labeling.

That degradation played a minor role in determining the differences in phospholipid composition is shown in Table 1. Although there was variability between phospholipid classes, the same class was degraded at a similar rate in the BBM and BLM except for PI. The reason(s) for this discrepancy was not evaluated in these studies.

These data, therefore, support the concept that phospholipids are synthesized and undergo spontaneous transfer to their final destination. They are in general agreement with the spontaneous phospholipid transfer theory [3, 4, 10–13, 15, 21, 23] rather than the multistep process of membrane flow [18]. Clearly (Figs. 2–4, Table 2), phospholipid classes are incorporated into the surface membrane at highly variable rates. How the cell regulates the process of phospholipid transfer from its site of synthesis to its destination are interesting and complex questions that could not be determined in the present studies.

In summary, we have demonstrated the large differences in BBM and BLM phospholipid composition in renal proximal tubule cells are largely determined by differences in the rate of phospholipid translocation to these membranes. Since phospholipids sorted to each membrane share a common synthetic site, and have similar degradation rates, cellular translocation processes beyond the synthetic step must be involved in the regulation of membrane phospholipid composition.

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