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Asymmetric distribution of gangliosides in rat renal brush-border and basolateral membranes

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Highly enriched brush-border and basolateral membranes isolated from rat renal cortex were used to study the distribution of endogenous gangliosides in the two distinct plasma membrane domains of epithelial cells. These two membrane domains differed in their glycolipid composition. The basolateral membranes contained more of both neutral and acidic glycolipids, expressed on a protein basis. In both membranes, the neutral glycolipids corresponding to mono-, di-, tri- and tetraglycosylceramides were present. The basolateral membranes contained more diglycosylceramide than the brush-border membranes. The major gangliosides found were G_{M4}, G_{M3}, and G_{D3} with minor amounts of G_{M1} and G_{D1a}. The latter were identified and quantified by sensitive iodinated cholera toxin binding assays. When the distribution of individual gangliosides was calculated as a percent of total gangliosides, the brush-border membranes were enriched with G_{M3}, G_{M1} and G_{D1a} compared to the basolateral membranes, which were enriched with G_{D3} and G_{M4}. The observation of a distinct distribution of glycolipids between brush-border and basolateral membranes of the same epithelial cell suggests that there may be a specific sorting and insertion process for epithelial plasma membrane glycolipids. In turn, asymmetric glycolipid biogenesis may reflect differences in glycolipid function between the two domains of the epithelial plasma membrane.

Introduction

The primary function of epithelial cells is to provide for and to regulate the unidirectional transport of various compounds. This is accomplished by having a polarized surface membrane separated by tight junctions into apical and basolateral membranes. In renal proximal tubule cells, these two membrane domains are different with respect to morphology, transport processes, ionic permeability, distribution of enzymes and sensitivity to hormones and drugs [1–3]. Apical (brush-border) membranes are associated with activities involved in the uptake of substances from the lumen. In contrast, adenylate cyclase, ($Na^+ + K^+$)-ATPase, and several hormone receptors have been identified as components of the basolateral (serosal) membrane which is in contact with the internal milieu facing the bloodstream [2–6]. These distinct functions are correlated with asymmetric

Abbreviations: G_{M4}, II^{3}NeuAc-GalCer; G_{M3}, II^{3}NeuAc-LacCer; G_{D3}, (II^{3}NeuAc)$_2$-LacCer; G_{M2}, II^{3}NeuAc-GgOse$_3$Cer; G_{M1}, II^{3}NeuAcGgOse$_4$Cer; G_{D1a}, IV^{3}NeuAc,-II^{3}NeuAc-GgOse$_4$Cer; GalCer, galactosylceramide; GlcCer, glucosylceramide; LacCer, lactosylceramide; GbOse$_3$Cer, Gal α1-4Galβ1-4Glcβ1-1′Cer; GbOse$_4$Cer, GalNacβ1-3Galα1-4Galβ1-4Glcβ1-1′Cer.

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distribution of protein and lipid components [7–9].

Although there is considerable information concerning the distribution of phospholipids [9] and individual proteins which mediate specific functions in each membrane [7,8,10,11], relatively little is known about the distribution of glycolipids. Recently, it has been shown that the glycolipid, Forssman antigen, is mostly localized in the apical membrane of one subline of Madin-Darby canine kidney cells [12]. In addition, changes in sialoglycolipid (ganglioside) composition on the apical membrane altered Na^+ transport across epithelial membranes [13]. The present studies were undertaken to determine the composition of glycolipids and, in particular, the gangliosides, in rat renal cortical brush-border (apical) and basolateral membranes. The differences we found in the distribution of glycolipids suggest that the biogenesis of these membrane components may involve asymmetric processing and sorting.

Materials and Methods

Silica gel 60-coated glass plates and aluminum sheets were from E. Merck through local distributors. *Vibrio cholerae* sialidase (EC 3.2.1.18) and cholera toxin were obtained from Calbiochem-Behring Corp., La Jolla, CA. ^{125}I-labeled cholera toxin was prepared using the chloramine-T procedure [14]. Protease (Pronase Type VI) was obtained from Sigma Chemical Co. G_{M3} and G_{M2} were from dog erythrocytes and Tay-Sachs brain, respectively; G_{M1} and G_{D1a} were from bovine brain; and G_{D3} was from egg yolk, all isolated as described previously [15]. Other glycolipid standards were from Supelco, Bellefonte, PA.

Isolation and characterization of plasma membrane fractions

The renal brush-border and basolateral membranes were prepared from cortical homogenates as described previously [16,17]. The quality of the membrane preparations was evaluated by enzyme markers. The specific activity of maltase, a brush-border membrane marker, in the cortical homogenate was 0.28 μmol/min per mg of protein; in the brush-border and basolateral membrane preparations, the specific activities were 2.95 and 0.09 μmol/min per mg of protein, representing enrichment factors of 11 and 0.3, respectively. The specific activity of $(Na^+ + K^+)$-ATPase, a basolateral membrane marker, in the cortical homogenate was 0.13 μmol/min per mg of protein; in the basolateral and brush-border membrane preparations, the specific activities were 1.31 and 0.06 μmol/min per mg of protein, representing enrichments factors of 10 and 0.46, respectively. In addition, the basolateral membrane preparations had virtually no transport activity characteristic of the brush-border preparation, e.g., Na^+-dependent D-glucose uptake and amiloride-sensitive Na^+-H^+ exchange. Furthermore, both the brush-border and basolateral membrane preparations presumably had little or no contamination by internal membranes, as enzyme markers of intracellular organelles show minimal activity [2,17]. It should be noted that the activity of the enzyme markers of intracellular organelles were not measured in the same preparations that were used for glycosphingolipid isolation, since large quantities of the membranes were required for the isolation and quantitation of the glycolipids. However, other brush-border and basolateral membrane preparations which had essentially the same enrichment factors in maltase and $(Na^+ + K^+)$-ATPase as the preparations used in the present studies had little or no contamination by the cytosol, mitochondria and lysosomes, as indicated by lactic dehydrogenase, cytochrome *c* oxidase and acid phosphatase activities, respectively [2,17].

Isolation and analysis of glycosphingolipids

Brush-border and basolateral membranes (up to 10 mg of protein) were sonicated in 2 ml of distilled water to make a homogeneous suspension and a portion was removed for protein determination. The lipids were extracted with 8 ml of chloroform/methanol (1 : 2, v/v) at 37 °C for 1 h. After centrifugation, the residue was washed with 4 ml of the same solvent. The combined extracts were taken to dryness, desalted on Sephadex G-25 columns, and separated into neutral and acidic fractions on DEAE-Sephadex as described previously [18]. The neutral fraction (solvent A) was applied to a Unisil column [19]. The eluted glycolipid fraction was treated with 0.2 N NaOH in chloroform/methanol (2 : 1, v/v) to hydrolyze phospholipids and was then neutralized with acetic

acid. After desalting, the solution was partitioned and the resultant lower phase was washed once with theoretical upper phase [20]. The neutral glycolipids were separated by thin-layer chromatography on silica gel with the solvent system chloroform/methanol/water (70 : 22 : 3, v/v). The neutral glycosphingolipids were detected by spraying the plates with a solution of 0.5% orcinol in ethanol/concentrated H_2SO_4 (8 : 2, v/v), heating at 110 °C for 10 min, and quantified by scanning densitometry at 430 nm. Gangliosides were isolated from the acidic fraction (solvent B) following alkaline hydrolysis, desalting on Sephadex G-25, and column chromatography on Unisil [18]. The latter was eluted first with chloroform/methanol (9 : 1, v/v) to increase the recovery of G_{M4} and G_{M3} in the chloroform/methanol (2 : 3, v/v) fraction [21]. The gangliosides were separated by thin-layer chromatography in chloroform/methanol/0.25% $CaCl_2$, visualized by resorcinol reagent, and quantified by scanning densitometry [18].

Sialidase treatment of gangliosides

Gangliosides isolated from 2 mg of basolateral membrane protein were incubated with 0.1 unit of *V. cholerae* sialidase in 1 ml of 0.1 M sodium acetate buffer containing 0.15 M NaCl and 9 mM $CaCl_2$ for 3 h at 37 °C. Chloroform/methanol (2 : 1, v/v) was added to give a final ratio of chloroform/methanol/buffer of 60 : 30 : 4.5, (v/v) and the sample was desalted on a Sephadex G-25 column. Glycolipids were separated by thin-layer chromatography on silica gel-coated glass plates in the solvent chloroform/methanol/0.25% $CaCl_2$ (60 : 35 : 8, v/v). Detection was with orcinol.

Analysis of gangliosides by overlay techniques

In order to identify gangliosides of the ganglliotetrose series, a newly developed overlay technique was used [13]. Briefly, gangliosides purified as described above were separated on aluminum-backed silica gel sheets using chloroform/methanol/0.2% $CaCl_2$ (5 : 4 : 1, v/v). After drying in air, the chromatogram was rapidly dipped twice in 0.4% polyisobutylmethylacrylate in hexane and air dried. The chromatogram was sprayed with and then soaked in 100 mM sodium acetate buffer (pH 5.5) containing 150 mM NaCl and 9 mM $CaCl_2$. The chromatogram was drained and overlaid with the same buffer containing sialidase (0.1 units/ml). After 2 h at 25 °C, the chromatogram was drained, dipped in four successive changes of the same buffer (ice-cold) and air dried. Then, the chromatogram was sprayed with and soaked in 50 mM Tris-HCl (pH 7.4)/150 mM NaCl/1% bovine serum albumin, drained and overlaid with the same solution containing ^{125}I-labeled cholera toxin (0.45 μCi/ml). After 30 min at 4 °C, the chromatogram was extensively washed with the same solution (ice-cold and lacking bovine serum albumin). The chromatogram was finally air dried and the bound toxin detected by autoradiography using Kodak X-Omat AR-2 film.

Assay of cholera toxin receptors

Binding of cholera toxin to brush-border and basolateral membranes was measured as described previously [22]. Briefly, membranes were incubated in 0.2 ml containing 25 mM Tris-HCl (pH 7.4)/127 mM NaCl/1 mM EDTA/0.1% bovine serum albumin/0.11 μCi ^{125}I-labeled toxin (0.48 μCi/pmol) for 45 min at 37 °C. Samples were then filtered under vacuum on 0.2 μ Millipore EGWP filters. Non-specific binding was determined in the presence of 200 nM unlabeled toxin. Binding of ^{125}I-labeled toxin was proportional up to 25 μg of membrane protein. Where indicated, basolateral and brush border membranes were incubated with protease or 0.1 U sialidase as described [23].

Results

Characterization of neutral glycolipids isolated from brush-border and basolateral membranes

The lipid extracts from brush-border and basolateral membranes were separated into neutral and acidic lipids and the glycosphingolipids were purified from each fraction and quantified. There was a greater amount of neutral glycosphingolipids in the basolateral membranes as compared to brush-border membranes, when expressed on a protein basis, 6.3 vs. 4.0 μg per mg protein (Table I). As shown in Fig. 1, the predominant neutral glycolipids from both membranes migrate on thin-layer cromatograms with mobilities identical to GlcCer plus GalCer (G_{L1}), LacCer (G_{L2}),

TABLE I

COMPOSITION OF NEUTRAL GLYCOLIPIDS OF BRUSH-BORDER AND BASOLATERAL MEMBRANES

Neutral glycolipids were isolated from the membranes, separated by thin-layer chromatography, detected with orcinol reagent and quantified by scanning spectrodensitometry as described in Materials and Methods. Values are the means ± S.E. of three separate preparations.

Glycolipid	μg/mg protein		% of total glycolipids	
	brush-border	basolateral	brush-border	basolateral
G_{L1}	1.70±0.13	2.50±0.19	43	39.5
G_{L2}	0.37±0.04	1.02±0.15	9	16
G_{L3}	0.80±0.09	1.05±0.16	20	16.5
G_{L4}	1.10±0.15	1.80±0.19	28	28

$GbOse_3Cer$ (G_{L3}), and $GbOse_4Cer$ (G_{L4}) *. The distribution of individual neutral glycolipids in the brush-border and basolateral membranes was similar (Table I). The major exception was that the basolateral membranes had an increased proportion of G_{L2} compared to brush-border membranes.

Characterization of gangliosides isolated from Brush-border and basolateral membranes

The basolateral membranes had a 2-fold higher content of gangliosides than did the brush-border membranes, 2.27 vs. 0.82 nmol lipid-bound sialic acid per mg protein (Table II). The major gangliosides were tentatively identified based on their relative chromatographic mobility and their sensitivity to bacterial sialidase as G_{M4}, G_{M3} and G_{D3} (Fig. 2). Following treatment of gangliosides isolated from the basolateral membranes with sialidase, several new resorcinol-negative bands with more rapid mobility were observed. Analysis indicated that these orcinol-positive bands corresponded to GalCer and LacCer (Fig. 3); the expected products of sialidase treatment of G_{M4}, G_{M3} and G_{D3}. No sialidase-resistant gangliosides were observed. As shown in Table II, all of the

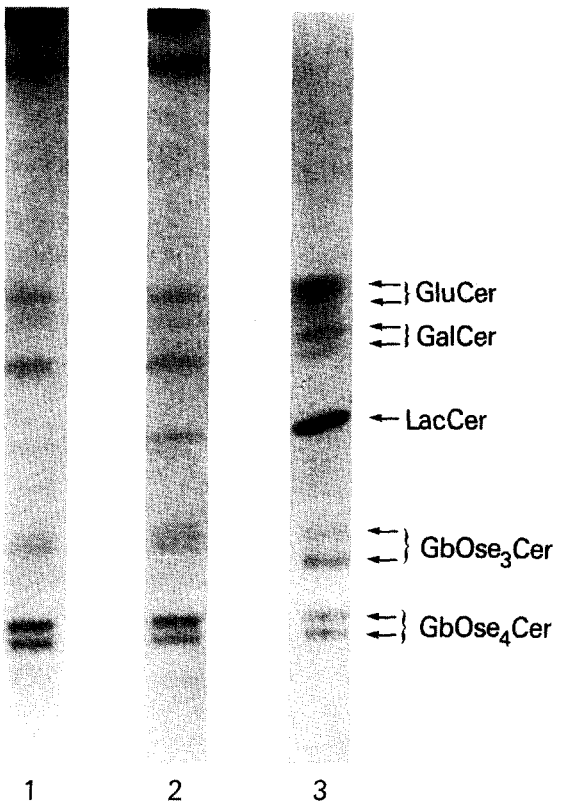

Fig. 1. Thin-layer chromatogram of neutral glycolipids extracted from brush-border and basolateral membranes. Neutral glycolipids were isolated from membranes, separated on silica gel-coated glass plates, and visualized with orcinol as described in Materials and Methods. Lane 1, neutral glycolipids extracted from 2 mg of brush-border membrane protein. Lane 2, neutral glycolipids extracted from 2 mg of basolateral membrane protein. Lane 3, neutral glycolipid standards. Arrows indicate the mobilities of the standard glycolipids. The origin is at the bottom of the figure.

individual gangliosides were found in both brush-border and basolateral membranes, but there appeared to be distinct differences in their relative distribution. G_{M3} was more enriched on the brush-border surface, but was also present in high amounts on the basolateral surface. The basolateral surface contained 7–8-times as much G_{D3} and G_{M4} on a protein basis as compared to the brush-border surface.

Gangliosides corresponding to G_{D1a} and G_{M1} were present in trace amounts, the latter being barely detectable by resorcinol spray. As the limit

* It should be noted that some glycolipids resolve into doublets on thin-layer chromatograms due to heterogeneity in the ceramide moiety [24].

TABLE II

GANGLIOSIDE COMPOSITION OF BRUSH-BORDER AND BASOLATERAL MEMBRANES

Gangliosides were separated and analyzed as described in Materials and Methods. Values are the means ± S.E. of three separate preparations. n.d., not detected in quantifiable levels; tr., trace.

Ganglioside	nmol sialic acid/mg protein		% of total ganglioside sialic acid	
	brush-border	basolateral	brush-border	basolateral
G_{D1a}	0.03 ± 0.004	0.04 ± 0.005	3.6	1.7
G_{D3}	0.08 ± 0.009	0.54 ± 0.042	9.8	23.8
G_{M1}	tr.	tr.	n.d.	n.d.
G_{M3}	0.63 ± 0.043	1.17 ± 0.092	76.8	51.6
G_{M4}	0.08 ± 0.003	0.52 ± 0.044	9.8	22.9

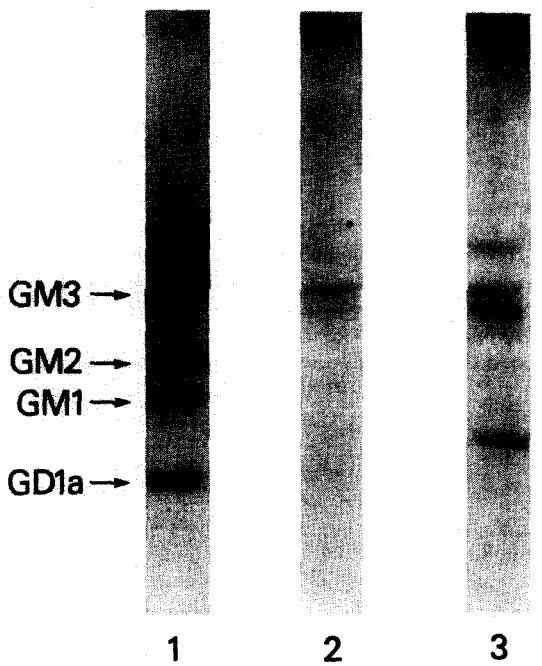

Fig. 2. Thin-layer chromatogram of gangliosides extracted from brush-border and basolateral membranes. Gangliosides were isolated from membranes, separated on silica gel-coated glass plates, and visualized with resorcinol as described in Materials and Methods. Lane 1, ganglioside standards. Lane 2, gangliosides extracted from 2 mg of brush-border membrane protein. Lane 3, gangliosides extracted from 2 mg of basolateral membrane protein. Arrows indicate the mobilities of the standard gangliosides.

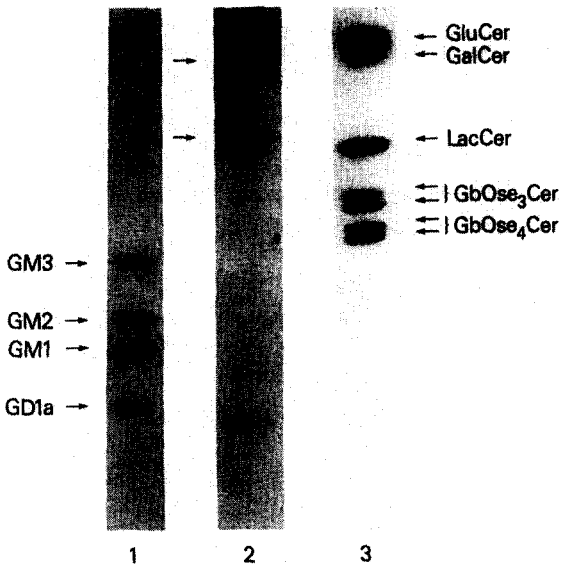

Fig. 3. Thin-layer chromatogram of sialidase-treated gangliosides extracted from basolateral membranes. Gangliosides were isolated from membranes, treated with sialidase, purified again, separated on silica gel-coated glass plates, and visualized with orcinol as described in Materials and Methods. Lane 1, ganglioside standards. Lane 2, glycolipids from sialidase-treated gangliosides isolated from 2 mg of basolateral membrane protein. Lane 3, neutral glycolipid standards. Arrows indicate the mobilities of the glycolipids.

of detection of gangliosides by resorcinol is 0.05 nmol of sialic acid [18], the more sensitive overlay technique was applied to detect the minor gangliosides corresponding to G_{M1} and G_{D1a} in brush-border and basolateral membranes. The purified gangliosides isolated from brush-border and basolateral membranes were separated by thin-layer chromatography and the chromatogram was overlaid with *V. cholerae* sialidase which converts the more complex gangliosides to G_{M1}. The latter G_{M1} can then be detected by overlaying the chromatogram with iodinated cholera toxin (Fig. 4). The iodotoxin bound to bands corresponding to G_{M1} and G_{D1a}, but not to bands corresponding to G_{M2} and G_{M3} (Fig. 4, lane 1). By this method of analysis, it is clear that gangliosides isolated from brush-border and basolateral membranes contained components corresponding to both G_{D1a} and G_{M1} (Fig. 4, lanes 2, 3). There was more G_{D1a} in both membrane preparations than G_{M1}. Only

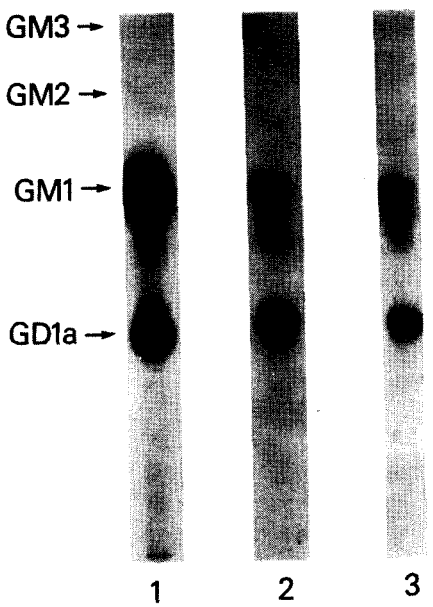

Fig. 4. Detection of G_{M1} and G_{D1a} gangliosides from brush-border and basolateral membranes by an overlay technique. The ganglioside fractions isolated from a portion equivalent to 0.5 mg of membrane protein were separated by thin-layer chromatography on an aluminum-backed silica gel sheet as described in Materials and Methods. The chromatogram was overlaid first with neuraminidase and then with ^{125}I-labeled cholera toxin. The bound toxin was detected by autoradiography (18 h exposure). Lane 1, ganglioside standards (0.5 nmol of each). Lane 2, gangliosides isolated from brush-border membranes. Lane 3, gangliosides isolated from basolateral membranes. Arrows indicate the location of G_{M3}, G_{M2}, G_{M1} and G_{D1a} as detected by orcinol spray.

one toxin-binding component, corresponding to G_{M1}, was detected in the ganglioside fractions when the chromatograms were not pretreated with sialidase (data not shown).

Although the amount of G_{M1} was too small to determine by the previous techniques, it could be estimated by ^{125}I-labeled cholera toxin binding to the isolated membranes. The basolateral and the brush-border membranes bound 1.9 and 2.2 pmol of iodotoxin per mg protein, respectively (Table III). In agreement with previous studies [23], protease treatment had no effect on the binding, whereas extraction of the membranes with mixtures of chloroform and methanol reduced cholera toxin binding by 90%. Treatment of the membranes with neuraminidase enhanced toxin binding over 4-fold, thus indicating that both membranes contained more of the complex gangliosides, such as G_{D1a}, than G_{M1}. As each toxin molecule could bind up to five molecules of ganglioside G_{M1} [25], the amount of toxin bound (Table III) could be used to estimate the relative amounts of G_{M1} and G_{D1a} in these membranes. The absolute amounts of G_{M1} and G_{D1a} were not significantly different between the basolateral and brush-border membranes. However, there were small differences in the distribution of G_{M1} and G_{D1a} when expressed as a percent of total gangliosides (calculated from Tables II and III). The brush-border membranes had a 3-fold greater proportion of both G_{M1} and G_{D1a} (1.3% and 4.2%, respectively) compared to basolateral membranes (0.4% and 1.3%, respectively).

TABLE III

BINDING OF IODINATED CHOLERA TOXIN TO BRUSH-BORDER AND BASOLATERAL MEMBRANES

Basolateral and brush-border membranes were incubated without or with protease or neuraminidase [23] and then 10 μg equivalents were assayed for binding of ^{125}I-labeled cholera toxin as described in Materials and Methods. In addition, membranes were delipidated as described, suspended in water with sonication and assayed for binding. Each value represents the mean of triplicate determinations which varied less than 10% and have been corrected for non-specific binding as measured in the presence of 0.2 μM unlabeled toxin.

Treatment	^{125}I-labeled cholera toxin bound (pmol/mg protein)	
	brush-border	basolateral
Control	2.2	1.9
Protease	2.3	2.1
Sialidase	9.1	7.9
Delipidation	0.23	0.21

Discussion

The plasma membrane components of epithelial cells are asymmetrically distributed between the apical and basolateral surfaces [11,26–28]. To study the distribution and polarity of endogenous glycosphingolipids in epithelial cells, we used highly enriched brush-border and

basolateral membranes isolated from renal proximal tubule cells [9,16,17]. This allows a direct comparison between brush-border and basolateral membranes isolated from the same cell homogenate. Contamination of either membrane fraction by other intracellular organelles is minimal. In addition, it is well known the bulk of the glycolipids are located in the plasma membrane. Consequently, minimal contamination by internal membranes would not significantly alter the observed glycolipid compositions of the brush-border and basolateral membrane fractions. Another advantage of this preparation is that rat kidney glycolipids have been extensively characterized by chemical analysis [29], methylation analysis [30–33], sequential degradation by enzyme treatment [30,32], NMR spectroscopy [34], negative ion fast atom bombardment mass spectroscopy [32], immunological techniques [32], and high-performance liquid chromatography [35–37]. The structure of these glycolipids were verified in this study through co-migration on thin-layer chromatography, sialidase treatment and cholera toxin binding.

In the present study, we found that the two membranes differed in their glycolipid pattern. The basolateral fraction had a larger content of glycosphingolipids, both neutral and acidic, than the brush-border fraction, when compared on a protein basis. Previously, a careful study of the lipid composition of apical and basolateral domains was carried out by Brasitus and Schachter on intestinal cells [38]. When we recalculated their data on a protein basis instead of on a percentage of total lipids basis as they did, we found that they also demonstrated that there was a 2-fold higher concentration of glycolipids in the basolateral membrane of the intestinal cell compared to the apical membrane.

There were some differences in both the content and distribution of individual neutral glycolipids between the brush-border and basolateral membranes. The largest difference was in G_{L2} (LacCer); basolateral membranes had almost 3-fold more G_{L2} than brush-border and G_{L2} represented a larger proportion of total neutral glycolipids in basolateral membranes than in brush-border membranes (16.1% vs. 9.3%). In this regard, another glycolipid, the Forssman antigen, appears by immunofluorescence staining to be preferentially localized in the apical membrane of epithelial MDCK strain II cells [12]. We also observed differences in the content and distribution of the major gangliosides. The basolateral membranes were enriched in total gangliosides and contained over 6-fold more G_{M4} and G_{D3} compared to the brush-border membranes. When the distribution of the individual gangliosides are expressed as a percentage of the total gangliosides in each membrane, however, the brush-border membranes were enriched with G_{M3} compared to basolateral membranes which were enriched with G_{D3} and G_{M4}.

Similar results were obtained in a previous study [13] using a toad kidney epithelial cell line (A6 cells). Confluent A6 cells were exposed in situ to sodium periodate, under conditions which oxidized only cell surface sialic acid residues exposed on the apical surface, followed by reduction with NaB^3H_4. To label both apical and basolateral surface gangliosides, the cells were gently scraped from the dish before the labeling procedure. All of the gangliosides were found on both the apical and basolateral surfaces, but there appeared to be some differences in their relative distribution. G_{M3} was the most enriched and G_{D3} the least enriched on the apical surface of these cells. Because of the possibility that some of the basolateral gangliosides may have become labeled due to the leakage of periodate and borohydride applied to the apical membrane, the differences in distribution might have been underestimated.

To identify the minor gangliosides, G_{D1a} and G_{M1}, two methods were used. In the first, gangliosides isolated from membrane fractions were separated by thin-layer chromatography and the chromatogram overlaid with sialidase, and then with ^{125}I-labeled cholera toxin. With this technique, it was clear that both brush-border and basolateral membranes contained more G_{D1a} than G_{M1}. However, the absolute amounts could not be determined quantitatively with this method because there was not a linear correlation between the concentration of G_{M1} on the plate and the density of the autoradiographic spot. To quantify the amounts of G_{M1} and G_{D1a} in these two membrane fractions, we used a cholera toxin binding assay with the isolated membranes. Cholera toxin

binds only to G_{M1} [25]; by treating the membranes with sialidase, we also were able to quantify the amount of G_{D1a}. Treatment of the membrane fractions with protease prior to cholera toxin binding ensured that the cholera toxin was not binding to glycoproteins and that the G_{M1} and G_{D1a} were not cryptic or masked. The absolute amounts of G_{M1} and G_{D1a} were essentially the same in the basolateral and brush-border membranes. On a percentage basis, the brush-border membrane appeared to have slightly more G_{M1} and G_{D1a} than the basolateral membrane. The differences found in the distribution of these gangliosides between the two distinct domains of the epithelial plasma membrane may lend support to our previous hypothesis that these specific gangliosides, G_{M1} and G_{D1a}, on the apical surface of A6 epithelia may be involved in the regulation of epithelial sodium transport [13]. However, the possibility that the small enrichment of G_{M1} and G_{D1a} could be due to the lack of G_{M4} and G_{D3} in the brush-border fraction cannot be excluded.

The observation of an unequal distribution of different glycolipids between brush-border and basolateral membranes indicates the existence of specific sorting and insertion processes for epithelial plasma membrane glycolipids. Previously using both A6 and MDCK epithelial cells, it was shown that gangliosides inserted into the apical membrane were unable to diffuse past the tight junctions to the basolateral surface [39]. Furthermore, when the apical surface of A6 cells was exposed to sialidase, the newly generated, but endogenous G_{M1} remained in the apical membrane. Of particular interest was the observation that the apically inserted gangliosides underwent internalization, yet did not appear on the basolateral surface [39]. Thus, the observed differences in ganglioside distribution most likely arise during membrane biogenesis.

A sorting mechanism for membrane glycoproteins is known [10,11]. Influenza virus, simian virus 5, and Sendai virus bud exclusively from the apical surface of MDCK cells, while vesicular stomatitis virus buds only from the basolateral membrane [11,40,41]. It was shown that the spike glycoproteins of these enveloped viruses generally did not appear at the inappropriate plasma membrane domain in infected cells throughout the course of their postsynthetic processing [11,40,41]. Similarly, it was found recently that an authentic membrane protein, the $(Na^+ + K^+)$-ATPase, was delivered directly to the basolateral domain without appearing, even transiently, on the apical surface [10]. Further work showed that sorting of these proteins occurred after passage through the *trans*-Golgi compartment and prior to cell surface delivery [42–44]. Recently, a 184 kDa membrane protein, which is an apical membrane marker, has been shown to be restricted to the apical pole when MDCK cells were grown under conditions in which tight junctions were absent [45]. Under the same conditions, a 63 kDa basolateral protein marker was not restricted to the basolateral surface. This implies that intradomain restriction mechanisms independent of tight junctions, such as self-aggregation or specific interactions with the cytoskeleton may be important in the regionalization of membrane components [45]. The question as to whether similar sorting mechanisms operate on glycolipids merits further study.

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