Effect of Golgi membrane phospholipid composition on the molecular species of GM3 gangliosides synthesized by rat liver sialyltransferase

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Abstract Previous studies have demonstrated that there is a change in the molecular species specificity of CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase (LacCerα2,3-ST) when the lipid composition of the Golgi membrane is altered (Kadowaki, Grant, and Williams. 1993. *J. Lipid Res.* **34:** 905–914). To understand the basis of this phenomenon, the molecular species specificity of rat liver LacCerα2,3-ST was determined under conditions in which the phospholipid class composition of the Golgi membrane was changed to resemble that of cultured mouse neuroblastoma NB2a cells, a cell line in which LacCerα2,3-ST exhibits no molecular species specificity. The change in the lipid composition of the Golgi membrane was accomplished by incubating the Golgi membrane vesicles with nonspecific lipid transfer protein and a 10-fold excess of liposomes prepared with various proportions of purified rat liver lipids. The molecular species specificity of LacCerα2,3-ST was also determined under conditions where the phospholipid molecular species composition but not the phospholipid class composition of the Golgi membrane was changed, and in which both the phospholipid class and molecular species compositions were changed by using liposomes prepared with lipids purified from a mouse brain tumor (ependymoblastoma). When using liposomes prepared with rat liver lipids, a change in the phospholipid class composition of the Golgi membrane to a composition similar to that of NB2a cells increased rather than decreased the molecular species specificity of rat liver LacCerα2,3-ST. On the other hand, a change in the molecular species composition of the phospholipids (i.e., using liposomes prepared with brain tumor lipids) essentially abolished the molecular species specificity of liver LacCerα2,3-ST whether the phospholipid class composition was the same as that of liver Golgi or NB2a cells. These results indicate that while changing the phospholipid class composition of the liver Golgi membrane did result in changes in the molecular species specificity of LacCerα2,3-ST, these changes did not explain the lack of specificity observed in NB2a cells. Rather, the lack of molecular species specificity of LacCerα2,3-ST observed in NB2a cells is due to the acyl group structure of the neuroblastoma phospholipids.—**Kadowaki, H., M. A. Grant, and T. N. Seyfried.** Effect of Golgi membrane phospholipid composition on the molecular species of GM3 gangliosides synthesized by rat liver sialyltransferase. *J. Lipid Res.* 1994. **35:** 1956–1964.

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The conformation (1, 2), activity (3–10), and specificity (11–13) of membrane enzymes can be modified by altering the membrane lipid composition. Consequently, there has been frequent speculation that changes in the membrane lipid composition may serve to regulate membrane enzymes in vivo. CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase (EC 2.4.99.9) (LacCerα2,3-ST) is an intrinsic Golgi membrane enzyme that catalyzes the synthesis of GM3 ganglioside, the major glycosphingolipid of most extraneuronal tissues. It has previously been reported that the activity and lactosylceramide (LacCer) molecular species specificity of LacCerα2,3-ST changed when the lipid composition of the membrane where the enzyme was located was altered (14). Specifically, when the LacCer molecular species specificity of rat liver LacCerα2,3-ST was determined, the enzyme displayed a distinct LacCer molecular species specificity (15). When, on the other hand, the molecular species specificity of LacCerα2,3-ST was examined in cultured mouse neuroblastoma NB2a cells (a cell line in which all the glycosphingolipids have a similar molecular species pattern (16)), the enzyme exhibited no LacCer molecular species specificity (14). When the lipid composition of NB2a cell microsomes was changed to resemble the lipid composition of rat liver Golgi membranes by incubating NB2a cell

Abbreviations: LacCerα2,3-ST, CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase; NeuAc, N-acetylneuraminic acid; LacCer, Galβ1-4Glc-ceramide; GM3, NeuAcα2-3Galβ1-4Glc-ceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; RRT, relative retention time; HPLC, high performance liquid chromatography. The ceramide molecular species abbreviations suggested by Breimer et al. (32) are used. For example, in the notation d18:1–18:0, the d18:1 represents the long chain base sphingosine (D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene) and 18:0 represents the fatty acid (octadecanoic acid).

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microsomes with nonspecific lipid transfer protein and a 10-fold excess of liposomes prepared with rat liver Golgi lipids, then NB2a cell LacCerα2,3-ST displayed the same distinct LacCer molecular species specificity as rat liver LacCerα2,3-ST. Similarly, when the lipid composition of rat liver Golgi membranes was altered to resemble the lipid composition of NB2a cells, then the molecular species specificity of rat liver LacCerα2,3-ST resembled that of the NB2a cells, i.e., there was no molecular species specificity.

The major difference in the phospholipid composition of rat liver Golgi and cultured NB2a cells was in the proportion of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (i.e., rat liver Golgi membranes contained 58% PC and 21% PE, while NB2a cells contained 78% PC and only 7% PE) (14). There was also a large percentage difference in the proportion of phosphatidylserine (PS), although the actual amount of PS was very low (i.e., rat liver Golgi membrane contained 3% and NB2a cells only 0.6% PS). The molecular species compositions of rat liver Golgi and NB2a cell phospholipids were also vastly different (14). In order to determine the basis for the difference in the molecular species specificity of rat liver and NB2a cell LacCerα2,3-ST, we have examined the LacCer molecular species specificity of rat liver LacCerα2,3-ST using liposomes prepared with various proportions of phospholipids purified from either rat liver or a mouse brain tumor.

EXPERIMENTAL PROCEDURES

Materials

[Sialic-4,5,6,7,8,9-^{14}C]cytidine 5'-monophosphate-neuraminic acid (326.5 mCi/mmol) was obtained from DuPont New England Nuclear (Boston, MA); cytidine 5'-monophospho-*N*-acetylneuraminic acid, *N*-palmitoyl-DL-dihydrolactocerebroside (d18:0-16:0 LacCer), *N*-stearoyl-DL-dihydrolactocerebroside (d18:0-18:0 LacCer), *N*-lignoceroyl-DL-dihydrolactocerebroside (d18:0-24:0 LacCer) were from Sigma (St. Louis, MO); cholesterol was from Nu-Chek-Prep (Elysian, MN); C18 solid extraction column (1 ml) was from Supelco (Bellefonte, PA). Analytical and HPLC grade solvents were obtained from Fisher (Medford, MA).

Preparation of Golgi membranes

The liver Golgi membrane was prepared from male Sprague-Dawley rats (Taconic Farms Inc., Germantown, NY) weighing 125-150 g as previously described (17).

Preparation of specific molecular species of LacCer

LacCer molecular species containing d18:1 or d20:1 long chain base coupled with either 16:0, 18:0, 20:0, 22:0, or 24:0 fatty acid were chemically synthesized by de- and re-acylation of LacCer prepared from bovine brain gangliosides (17). The purity of each LacCer molecular species was over 95% as determined by HPLC.

Preparation of liposomes

Lipids were extracted from perfused rat livers and mouse brain tumors (ependymoblastoma grown subcutaneously in the flank of the B6 mouse (18)) with chloroform-methanol 2:1 and partitioned with saline into two phases (19). The lower phase was separated into neutral lipids and individual phospholipid classes by HPLC (20). Phospholipid classes were quantitated by phosphorus analysis (21). The liposomal lipids were prepared with three different mixtures of lipids (**Table 1**). The purified phospholipid classes and cholesterol were mixed in the same proportion as they occur in the rat liver Golgi membrane (control liposomes), in the same proportion as they occur in rat liver Golgi membrane except that the proportion of PC and PE was the same as that of cultured mouse neuroblastoma NB2a cells (14) while the proportion of other lipids was maintained the same as in rat liver Golgi membrane (low-PE liposomes), or in the same proportion as the control liposomes but with the PS deleted (no-PS liposomes). Liposomes were prepared with 10 nmol of single molecular species of LacCer and 200 nmol of phospholipids as previously described (15).

LacCerα2,3-ST assay

LacCerα2,3-ST activity was determined in the presence of purified nonspecific lipid transfer protein (22) as previously described (17). [^{14}C]*N*-acetylneuraminic acid (NeuAc) incorporation into GM3 from endogenous substrates was determined with liposomes prepared without LacCer, and this endogenous activity was subtracted from the experimental values.

TABLE 1. Proportion of lipids in liposomes

Lipid	Liposomes		
	Control	Low-PE	No-PS
	mol %		
Phosphatidylcholine	57.8	72.7	59.9
Phosphatidylethanolamine	21.2	6.3	22.0
Phosphatidylinositol	8.1	8.1	8.1
Phosphatidylserine	2.9	2.9	-
Sphingomyelin	2.8	2.8	2.8
Cholesterol	7.2	7.2	7.2

Lipid composition of control liposomes is the same as in the rat liver Golgi membrane (14). Low-PE liposomes contain the same proportions of PC and PE observed in cultured mouse neuroblastoma NB2a cells (14) but with the other lipids maintained in the same proportion as in the liver Golgi membrane. No-PS liposomes contain the same proportion of phosphatidylinositol, sphingomyelin, and cholesterol as the control liposomes, but PS was deleted and the amount of both PC and PE was increased by 3.7% compared to the control liposomes.

Separation of Golgi membranes from liposomes and quantitation of LacCer molecular species

Liver Golgi membranes (100 μg of protein) were incubated with liposomes containing 4 nmol each of five molecular species of LacCer (i.e., d18:0-16:0, d18:0-18:0, d18:0-24:0, d18:1-18:0, and d20:1-18:0) in the presence of nonspecific lipid transfer protein (80 μg of protein) under the same incubation conditions as the LacCerα2,3-ST assay, except that CMP-NeuAc was omitted. Liposomes (400 nmol of phospholipids) were prepared with ependymoblastoma lipids mixed in the same proportion as in cultured NB2a cells (14). After 10, 30, and 60 min incubations at 37°C, Golgi membranes were separated from liposomes by ultracentrifugation as previously described (15). LacCer in the Golgi membranes was isolated (15), perbenzoylated (23), and quantitated by normal phase HPLC (24). The molecular species of LacCer were separated and quantitated by reversed phase HPLC (25).

Other analyses

The diradyl forms of PC and PE, and the molecular species of the phospholipid classes were determined by HPLC after hydrolysis by phospholipase C (26) and conversion of the resulting diglycerides to benzoyl esters (27). Ceramide molecular species of sphingomyelin were determined as previously described (26) except that the HPLC column (2 × 250 mm) was Spherisorb (3 μm, MetaChem Technologies, Redondo Beach, CA) and the mobile phase was methanol-acetonitrile 1:1. Protein was quantitated by the method of Lowry et al. (28) with bovine serum albumin as standard. Statistical differences between groups were determined by ANOVA with one-way analysis of variance and comparisons were made using the Fisher procedure at a significance of 0.05.

TABLE 2. Glycerophospholipid molecular species compositions of the incubation mixtures using liposomes prepared with rat liver lipids

Major Molecular Species	Control	Low-PE	No-PS
		mol %	
16:0-22:6	8.5	6.8	8.4
18:1-22:6, 16:0-16:1	2.1	2.3	2.2
16:0-20:4, 16:1-18:1	12.0	12.1	12.2
18:1-20:4	2.8	2.6	3.0
16:0-18:2	18.8	18.8	19.2
18:1-18:2	4.0	4.0	3.9
18:0-22:6, 16:0-16:0[a]	5.8	5.3	5.0
16:0-18:1	8.8	10.2	9.2
18:0-20:4, 18:1-18:1[b]	22.1	21.1	21.8
18:0-18:2	13.4	14.6	13.5
18:0-18:1	1.7	2.0	1.6

[a]18:0-22:6 and [b]18:0-20:4 are the major molecular species in these fractions for all the phospholipid classes of liver lipids. Molecular species are presented as less hydrophobic to more hydrophobic (from top to bottom). The liposomes were prepared with various proportions of lipids purified from rat liver as presented in Table 1. The distribution of molecular species is calculated from the molecular species composition of each phospholipid class (i.e., PC, PE, PI, and PS) in the liposomes. The molecular species contributed by the liver Golgi membrane (10% of lipids in the incubation mixture) are also included in the compositions of the low-PE and no-PS liposomes as the nonspecific lipid transfer protein in the assay system rapidly equilibrates these lipids between liposomes and Golgi membrane.

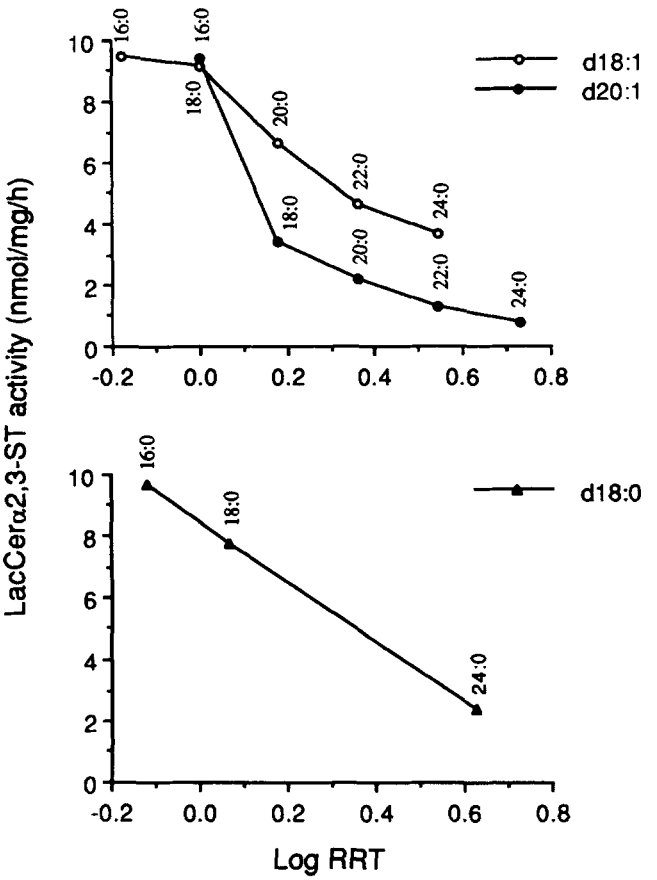

Fig. 1. The activity of LacCerα2,3-ST towards various molecular species of LacCer as a function of the log of the relative retention time (RRT) of LacCer. RRT is calculated by dividing the absolute retention time of each molecular species of LacCer by the retention time of d18:1-18:0 LacCer (15). The reaction mixture contained 10 nmol of a single molecular species of LacCer in control liposomes (200 nmol of phospholipids), 50 μg of Golgi membrane protein, 45-55 μg of purified nonspecific lipid transfer protein, 50 nmol of CMP-[^{14}C]NeuAc in a final volume of 50 μl and at a pH of 6.2. After incubation for 60 min at 37°C, [^{14}C]GM3 was determined (17). All the incubations were performed in duplicate (the range was within ± 7% of the indicated means) using the same Golgi membrane preparation. The long chain bases and fatty acids are shown in each panel.

RESULTS

The activity of rat liver LacCerα2,3-ST towards each of 13 molecular species of LacCer, containing either d18:1 or d20:1 long chain base coupled with 16:0, 18:0, 20:0, 22:0, or 24:0 fatty acid or d18:0 long chain base coupled with 16:0, 18:0, or 24:0 fatty acid, was determined with saturat-

ing concentrations of both LacCer and CMP-NeuAc. The LacCer was incorporated into liposomes prepared with purified rat liver lipids mixed in the same proportion as they occur in rat liver Golgi membranes (14) (Table 1, control liposomes), in that same proportion but with the amount of PC and PE adjusted to that of cultured mouse neuroblastoma NB2a cells (14) (low-PE liposomes), or in the same proportion as they occur in rat liver Golgi membranes except that PS was deleted (no-PS liposomes). In spite of the large difference in the ratio of PC and PE between control and low-PE liposomes, there were only minor differences in the acyl group composition of the liposome preparations (**Table 2**). Therefore, the variation in the activity of LacCerα2,3-ST among the three liposome preparations was probably due mainly to the different proportion of PC, PE, or PS (head groups).

With control liposomes, the activity of LacCerα2,3-ST towards all three molecular species with a 16:0 fatty acid but a different long chain base (i.e., d18:1–16:0, d18:0–16:0, and d20:1–16:0) was the same (**Fig. 1**). For those molecular species with a d18:1 long chain base, the activity of LacCerα2,3-ST was the same with 16:0 and 18:0 fatty acids, but with the longer chain fatty acids, the activity decreased as the carbon number of the fatty acid increased. However, the decrease was progressively less with each additional 2 carbons. For those molecular species with a d20:1 long chain base, the activity of LacCerα2,3-ST towards d20:1–18:0 was only a third of the activity towards d20:1–16:0, and with the longer chain fatty acids the activity of the enzyme also decreased as the number of carbons increased. As was the case with d18:1 long chain base, the decrease was progressively less with each additional 2 carbons. For those molecular species with a d18:0 long chain base, the activity of LacCerα2,3-ST decreased nearly linearly as the carbon number of the fatty acid increased.

With low-PE liposomes (**Fig. 2,** solid bars), the activity of LacCerα2,3-ST towards all the molecular species with a 16:0 fatty acid was similar and was nearly the same as that obtained with control liposomes. For those molecular species with a d18:1 long chain base, the activity of LacCerα2,3-ST with the longer chain fatty acids was less than that obtained with control liposomes, and the differences between the activity of LacCerα2,3-ST obtained with control and low-PE liposomes became progressively greater as the carbon number of the fatty acid increased. Likewise, for those molecular species with a d20:1 long chain base, the activity of LacCerα2,3-ST obtained with the longer chain fatty acids (18–24 carbons) was less with the low-PE liposomes than with control liposomes. However, in contrast to those molecular species with a d18 long chain base, the greatest difference between control and low-PE liposomes was obtained with d20:1–18:0 and d20:1–20:0. The difference between the activity of LacCerα2,3-ST obtained with control and low-PE liposomes became progressively less with a further increase in the carbon number of the fatty acid. For those molecular species with a d18:0 long chain base, the activity of LacCerα2,3-ST was generally similar to that obtained with the corresponding d18:1 molecular species.

With the no-PS liposomes (Fig. 2, dotted bars), the activity of LacCerα2,3-ST towards all the molecular species with a 16:0 fatty acid was also nearly the same as that obtained with control liposomes. For the other molecular species with a d18:1 long chain base, the difference in the activity of LacCerα2,3-ST between control and no-PS liposomes was one half of the difference between control and low-PE liposomes. For those molecular species with a

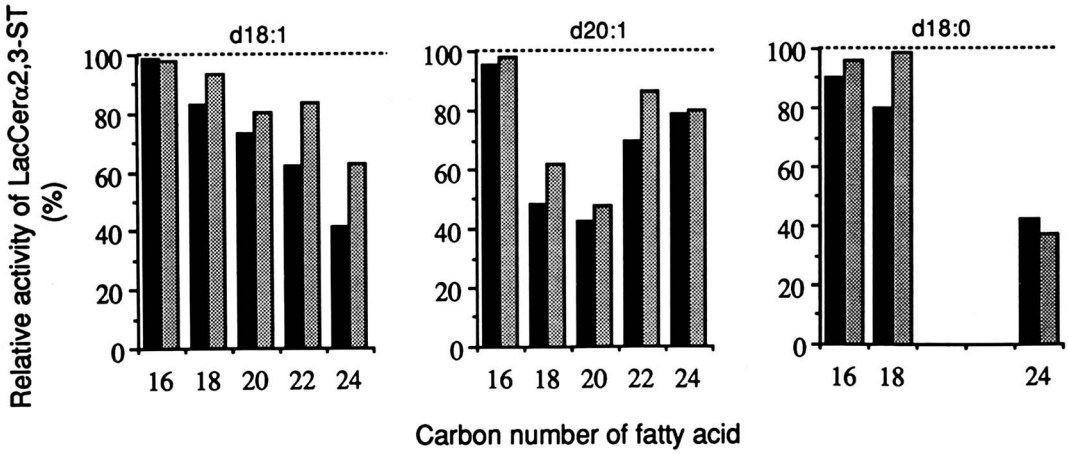

Fig. 2. The effect of low-PE and no-PS liposomes on the activity of LacCerα2,3-ST towards various molecular species of LacCer. The reaction conditions were the same as described in Fig. 1, except that liposomes were prepared with rat liver lipids mixed in the proportions shown in Table 1 for low-PE or no-PS liposomes. The Golgi membrane preparation was the same preparation used for the experiment presented in Fig. 1. Data are expressed as a percentage of the activity obtained with control liposomes for that particular molecular species of LacCer. All incubations were performed in duplicate (the range was within ± 10% of the indicated means). The long chain bases and fatty acids are indicated in each panel; solid bars, low-PE; dotted bars, no-PS.

d20:1 long chain base, the activity of LacCerα2,3-ST obtained with no-PS liposomes was similar to that obtained with low-PE liposomes. The activity of LacCerα2,3-ST towards d18:0–16:0 and d18:0–18:0 using no-PS liposomes was similar to that obtained with control liposomes, but the activity obtained with d18:0–24:0 was much lower than that with control liposomes and was the same as that obtained with low-PE liposomes.

In another experiment, the molecular species specificity of LacCerα2,3-ST was determined using a limited number of molecular species (d18:0–16:0, d18:0–18:0, d18:0–24:0, d18:1–18:0, and d20:1–18:0) incorporated into liposomes prepared with lipids purified from a mouse brain tumor (ependymoblastoma) grown subcutaneously in the flanks of mice. Tumor lipids were reconstituted in the same proportion as in rat liver Golgi (Table 1, control liposomes) or as in cultured mouse neuroblastoma NB2a cells (i.e., 77.7% PC, 6.7% PE, 4.5% PI, 0.6% PS, 3.6% sphingomyelin, and 6.9% cholesterol (14)). Unlike liver PC and PE, the PC and PE isolated from the tumor contained both alkenylacyl and alkylacyl as well as diacyl lipids (**Table 3**). Each tumor phospholipid class also had a radically different molecular species composition than the corresponding rat liver phospholipid. Consequently, the glycerophospholipid molecular species composition of the tumor liposome preparations (**Table 4**) was very different from the liposomes prepared with rat liver lipids (Table 2). Compared to liposomes prepared with rat liver lipids, the liposomes prepared with tumor lipids had much less of those phospholipid molecular species containing polyunsaturated fatty acids (22:6, 20:4 and 18:2) and much more of those molecular species containing fatty acids with either no or a single double bond (i.e., 16:0–16:0,[2] and especially 16:0–18:1 and 18:0–18:1). Of particular note was the large increase in those molecular species with two unsaturated fatty acids (i.e., 18:1–22:6, 18:1–20:4, and 18:1–18:1) in the tumor lipids. However, there were only small variations in the molecular species composition of the liposomes prepared with tumor lipids mixed in the liver Golgi proportion and in the NB2a cell proportion, except for 16:0–18:2, 16:0–18:1, and 18:1–18:1 (Table 4). The sphingomyelin molecular species composition of the tumor liposome preparations was also markedly different from the liposomes prepared with rat liver lipids (**Table 5**).

For those molecular species of LacCer containing a d18 long chain base, the activity of LacCerα2,3-ST obtained with ependymoblastoma lipid liposomes prepared in the liver Golgi proportion (**Fig. 3,** dotted bars) was generally lower than the activities obtained with control liposomes, i.e., with rat liver Golgi liposomes (solid bars). In contrast, the activity of LacCerα2,3-ST towards d20:1–18:0 was significantly higher than that obtained with control liver liposomes. When the activity of LacCerα2,3-ST was determined using liposomes prepared with tumor lipids mixed in the NB2a cell proportion (hatched bars), the activity with all the molecular species was about one half of the activity obtained with tumor liposomes mixed in the

TABLE 3. Distribution of the diradyl forms of PC and PE in the incubation mixtures

Phospholipid	Rat Liver	Ependymoblastoma	
		Liver Golgi Proportion	NB2a Cell Proportion
	mol %	*mol%*	
PC			
Alkenylacyl		1.3	1.7
Alkylacyl		5.4	7.3
Diacyl	62.3	56.9	74.5
PE			
Alkenylacyl	0.6	8.3	2.7
Alkylacyl		1.6	0.5
Diacyl	22.2	13.4	3.8

Data are presented as the percentage of the total phospholipids. The diradyl forms contributed by the liver Golgi membrane (10% of lipids in the incubation mixture) are also included in the compositions of the brain tumor incubation mixtures.

TABLE 4. Glycerophospholipid molecular species composition of the incubation mixtures using liposomes prepared with mouse brain tumor lipids

Major Molecular Species	Ependymoblastoma	
	Liver Golgi Porportion	NB2a Cell Proportion
	mol %	
16:0–22:6	3.5	3.3
18:1–22:6, 16:0–16:1	4.2	4.4
16:0–20:4, 16:1–18:1	7.9	8.0
18:1–20:4	4.6	4.2
16:0–18:2	10.3	11.7
18:1–18:2	5.8	6.1
18:0–22:6, 16:0–16:0[a]	6.9	6.5
16:0–18:1	18.3	22.0
18:0–20:4, 18:1–18:1[b]	20.0	17.0
18:0–18:2	9.9	9.3
18:0–18:1	8.6	7.5

[a]16:0–16:0 and [b]18:1–18:1 are the major molecular species in these fractions for all the phospholipid classes of the mouse brain tumor grown in the mouse flank. The molecular species contributed by the rat liver Golgi membrane (10% of lipids in the incubation mixture) are also included in the compositions.

[2]The notation used for glycerophospholipid molecular species is not intended to indicate the position of the fatty acid on the glycerol.

TABLE 5. Ceramide molecular species composition of sphingomyelin in the incubation mixtures

Molecular Species	Liver Golgi	Ependymoblastoma
	mol %	
d18:1–14:0		4.3
d18:1–16:0	14.0	46.7
d18:1–17:0	1.3	3.4
d18:1–18:0	9.5	7.5
d18:1–20:0	2.4	1.3
d18:1–22:0	10.3	4.9
d18:1–23:0	12.7	2.5
d18:1–24:0	29.8	8.8
d18:1–24:1	16.9	13.3
d18:1–24:2	3.0	7.3

The molecular species contributed by the liver Golgi membrane (10% of lipids in the incubation mixture) are also included in the compositions of the brain tumor incubation mixture.

liver Golgi proportion. Thus, compared to control liposomes, the most notable difference was the decrease in the activity of LacCerα2,3-ST towards those molecular species of LacCer containing a d18 long chain base with either 16:0 or 18:0 fatty acid.

In order to verify that the differences in the activity of LacCerα2,3-ST towards the various molecular species of LacCer obtained with liposomes prepared with liver or ependymoblastoma lipids were not due to differences in the amount of LacCer in the Golgi membrane, the amount of each of the five molecular species of LacCer employed in the experiment shown in Fig. 3 was determined in the Golgi membrane after 10, 30, and 60 min of incubation under the same conditions used for the LacCerα2,3-ST assay except that CMP-[^{14}C]NeuAc was omitted (**Table 6**). For this experiment liposomes were prepared with ependymoblastoma lipids mixed in the cultured NB2a cell proportion. The transfer of LacCer from the liposomes to the Golgi membrane was essentially completed in 10 min, and at all incubation times there were no significant differences in the proportion of the LacCer molecular species between the liposomes and the Golgi membranes except for d20:1–18:0 at 10 min. Ten min was chosen for the shortest incubation time because, in the enzyme assay system used in the present studies, the enzyme and liposomes were preincubated together in the presence of transfer protein for 10 min before the reaction was initiated by the addition of CMP-[^{14}C]NeuAc.

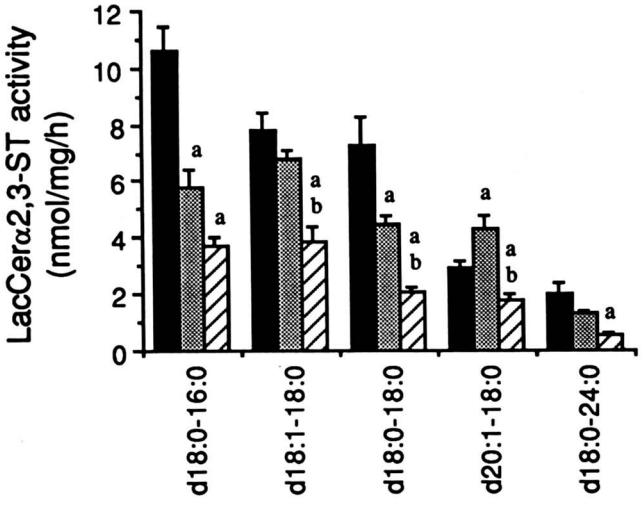

Fig. 3. The effect of mouse brain tumor lipids on the activity of the rat liver LacCerα2,3-ST. Liposomes were prepared with lipids purified from a mouse brain tumor, ependymoblastoma, grown subcutaneously in the mouse flank. Solid bars: liposomes prepared with liver lipids mixed in the same proportion as rat liver Golgi membranes (control liposomes). These data are from ref. 17. Dotted bars: liposomes prepared with tumor lipids mixed in the proportion as in control liposomes. Hatched bars: liposomes prepared with tumor lipids mixed in the same proportion as NB2a cell lipids. The results are mean ± SE of six determinations for liver lipid liposomes, and four determinations for tumor lipid liposomes. Statistically significant differences ($P < 0.05$) between the activity of LacCerα2,3-ST towards a given LacCer molecular species from (a) control liver lipid liposomes and (b) from tumor lipids mixed in control liposome proportions are indicated above the bars.

DISCUSSION

LacCerα2,3-ST is an intrinsic membrane enzyme with its catalytic domain facing into the lumen of the Golgi apparatus (29). The water-soluble substrate, CMP-NeuAc, is transported into the lumen of the Golgi by a membrane carrier protein (30). To ensure that the transport of CMP-NeuAc into the lumen of the Golgi is not the limiting factor

TABLE 6. Distribution of LacCer molecular species transferred to Golgi membrane fraction from ependymoblastoma lipid liposomes

Molecular Species	Incubation Time		
	10 min	30 min	60 min
	nmol		
d18:0–16:0	1.25 ± 0.07	1.24 ± 0.05	1.33 ± 0.11
d18:1–18:0	1.31 ± 0.10	1.25 ± 0.09	1.36 ± 0.12
d18:0–18:0	1.29 ± 0.08	1.27 ± 0.06	1.39 ± 0.09
d20:1–18:0	1.41 ± 0.08[a]	1.33 ± 0.09	1.40 ± 0.11
d18:0–24:0	1.27 ± 0.06	1.25 ± 0.05	1.30 ± 0.07
Total	6.52 ± 0.37	6.33 ± 0.33	6.72 ± 0.49

The incubation mixture contained 20 nmol (4 nmol of each molecular species listed in the table) of LacCer in liposomes (400 nmol lipid phosphorus), 100 μg of liver Golgi membrane protein, 80 μg of purified nonspecific lipid transfer protein at a final volume of 100 μl at pH 6.2. Liposomes were prepared with ependymoblastoma lipids mixed in the same proportion as cultured NB2a cell lipids. Incubations were performed at 37°C for the indicated times. Golgi membranes were separated from liposomes and LacCer was quantitated as described in Experimental Procedures. The results are mean ± SD of three determinations.

[a]Statistically significant differences ($P < 0.05$) from other molecular species in a given incubation time.

in the enzyme assay and to permit the direct transfer of the lipophilic substrate, LacCer, to the leaf of the Golgi membrane where the catalytic site of the enzyme is located, Golgi vesicles were converted into inside-out vesicles by sonication. GM3 synthesis is increased approximately 2.5-fold by sonication and the addition of detergents to the sonicated Golgi preparation does not further increase the activity of the enzyme (24). In the assay system used in this study, LacCer is transferred from liposomes to the Golgi membrane by nonspecific lipid transfer protein which also promotes the rapid equilibration of phospholipids and cholesterol (22, 31) between the liposomes and Golgi vesicles. Therefore, in the presence of a large excess (10-fold) of liposomal lipids, it is also possible to change the lipid composition of the membrane where LacCerα2,3-ST is located.

Changes in the phospholipid class composition of membranes or of detergent-solubilized membrane preparations have been shown to modify the activity of a number of intrinsic membrane enzymes (1–13), including LacCerα2,3-ST. In one such study, Burczak, Soltysiak, and Sweeley (5) studied the effect of adding exogenous phospholipids to detergent-solubilized LacCerα2,3-ST. In that study the addition of PS to the enzyme inhibited the enzyme activity, while the addition of PE had no effect and the addition of PC resulted in a large (10-fold) increase in LacCerα2,3-ST activity. This is almost the opposite of what was observed in the present study, where deleting PS resulted in a decrease in the activity of LacCerα2,3-ST towards most molecular species of LacCer, and where an increase in the proportion of PC at the expense of PE also resulted in a decrease in LacCerα2,3-ST activity. As neither the source of the enzyme, the substrate used, nor the acyl composition of the phospholipids was specified, it is difficult to determine what factors may be responsible for the difference in the results obtained in that study and the results of the present study.

In another study (6) examining intestinal Golgi LacCerα2,3-ST, it was observed that treating rats with dexamethasone for 4 days resulted in a 2-fold increase in the activity of LacCerα2,3-ST and a modest change in the fatty acid composition (i.e., an increase in 18:2 and 20:4 fatty acids), but no change in the phospholipid class composition of the Golgi membrane. Thus, the results of that study are in general agreement with the results of the present study in which the more polyunsaturated lipids (rat liver) gave a higher LacCerα2,3-ST activity. However, as the actual amount of LacCerα2,3-ST was not determined in that study, it is possible that the effect of dexamethasone on LacCerα2,3-ST activity could have been due to an increase in the amount of the enzyme.

In most mammalian tissues there are major differences in the ceramide molecular species composition among the various glycosphingolipid classes of the tissue, as well as differences between tissues. In contrast, in cultured mouse neuroblastoma NB2a cells (16), and a number of other cultured neuroblastoma cells (unpublished observations), all the glycosphingolipid classes have a similar ceramide molecular species composition. In previous studies (14) using the same assay system used in the present study and liposomes prepared with lipids extracted from rat liver Golgi membranes or cultured mouse neuroblastoma NB2a cells, it was observed that when the lipid composition of the membrane in which LacCerα2,3-ST was located was changed, the molecular species specificity of the enzyme was the same as that of the tissue from which the lipids were obtained rather than the tissue from which the enzyme was obtained. In that study, when using liposomes prepared with NB2a cell lipids, the activity of rat liver LacCerα2,3-ST was low and the enzyme had virtually no molecular species specificity. Thus, the absence of molecular species specificity observed when using NB2a cell lipids was due to a decrease in the activity of LacCerα2,3-ST towards those molecular species of LacCer containing a d18:1 or d18:0 long chain base and a 16:0 or 18:0 fatty acid compared to the activity of LacCerα2,3-ST towards the same molecular species determined with liposomes prepared with rat liver Golgi lipids.

In the present study, using purified rat liver lipids, a change in the phospholipid class composition of the Golgi membrane to a phospholipid class composition similar to NB2a cells (i.e., low-PE liposomes) had a substantial effect on the molecular species specificity of LacCerα2,3-ST, but the changes in no way explain the lack of molecular species specificity observed with NB2a cell lipids. In fact, with the low-PE (and the no-PS) liposomes, the molecular species specificity of LacCerα2,3-ST became more pronounced because there was little or no change in the activity of LacCerα2,3-ST towards d18:1–16:0 or d18:0–16:0, while there was a progressively greater decrease in the activity of the enzyme with the longer chain fatty acids. Because the results with the low-PE liposomes (in which there was a large decrease in the proportion of PE (70%) and a compensatory increase in PC (25%)) and with the no-PS liposomes (in which there was a mere 4% increase in both PC and PE) are so similar in kind, if not in degree, it is likely that the more pronounced molecular species specificity of LacCerα2,3-ST was the result of a specific interaction between the enzyme and the head group of the phospholipids and that the interaction involves the free amino group present in both of these phospholipids.

As the results with the low-PE or no-PS liposomes demonstrate that the absence of LacCer molecular species specificity of LacCerα2,3-ST in NB2a cells was not due to the phospholipid class composition of the cells, it seemed probable that the lack of LacCer molecular species specificity in NB2a cells was due to the molecular species

composition of the tumor phospholipids. Although there are some differences in the phospholipid molecular species composition of ependymoblastoma tumors grown in the mouse and that of cultured NB2a cells, these differences are small compared to the major differences in the phospholipid molecular species composition of the liver lipids and the ependymoblastoma lipids. (Cultured neuroblastoma NB2a cells contain even less polyunsaturated fatty acids, particularly 18:2, (14) than the ependymoblastoma tumor grown in the mouse.) When liposomes were prepared with lipids isolated from ependymoblastoma and reconstituted to the NB2a cell proportion (Fig. 3, hatched bars), the activity of rat liver LacCerα2,3-ST was similar to that observed with NB2a cell lipids. Specifically, d18:0–16:0, d18:1–18:0, d18:0–18:0, and d20:1–18:0 LacCer all had essentially the same activity. Even when the tumor lipids were mixed in the same proportion as in rat liver Golgi membranes (Fig. 3, dotted bars), LacCerα2,3-ST displayed very little molecular species specificity even though the activity was generally higher, because the activity of LacCerα2,3-ST towards d20:1–18:0 increased while the activity towards d18:0–16:0, d18:1–18:0 and d18:0–18:0 decreased. Similar results were observed when liposomes were prepared with another mouse brain tumor (CT2A) grown in a mouse flank, which has a similar proportion of diradyl forms of PC and PE, and a similar glycerophospholipid and sphingomyelin molecular species composition (data not shown). In all cases, including when using liposomes prepared with NB2a cell lipids (14), the activity of LacCerα2,3-ST towards d18:0–24:0 was less than towards the other molecular species.

One possible explanation for the difference in the activity of LacCerα2,3-ST when using liver lipids as opposed to neuroblastoma lipids is that there is a difference in the amount of LacCer molecular species transferred to the Golgi membrane. As shown in Table 6, the amount of LacCer transferred to liver Golgi membrane from tumor lipid liposomes mixed in the NB2a cell proportion was rapid and there was essentially no variation in the relative proportion of LacCer molecular species. Using nearly identical procedures and liposomes prepared with rat liver Golgi lipids, we have previously shown that the proportion of the LacCer molecular species transferred to the Golgi membrane was also the same as the proportion initially present in the liposomes (15). With the liver Golgi lipid liposomes there was initially a rapid transfer of LacCer to the Golgi membrane (approximately 60% of the total transfer occurs by 10 min) followed by a gradual increase in the amount transferred (24). At the end of the 60-min incubation, the total amount of LacCer (6.68 $\pm$ 0.87, mean $\pm$ SD of 18 determinations) transferred to the Golgi membrane from the liver lipid liposomes, which also contained 20 nmol of LacCer (15), was comparable to that transferred to the Golgi membrane from the ependymoblastoma lipid liposomes after 10 min (Table 6). Thus, the decrease in the activity of LacCerα2,3-ST obtained with the tumor lipid liposomes is not due to a decreased concentration of substrate in the Golgi membrane and is, therefore, probably due to a decrease in the intrinsic activity of the enzyme.

The changes in the LacCer molecular species specificity of LacCerα2,3-ST that occur when using liposomes prepared with the tumor lipids (ependymoblastoma, CT2A, or NB2a cells) were generally similar in kind whether the tumor lipids were mixed in the liver Golgi proportion (ependymoblastoma or CT2A) or in the NB2a cell proportion, but the total activity was less when the liposomes were prepared in the NB2a cell proportion. However, as there are only small differences in the phospholipid molecular species compositions between the liposomes prepared with brain tumor lipids mixed in the liver proportion and in the NB2a cell proportion, it is unlikely that the acyl group composition per se is the factor responsible for the difference in the total activity of LacCerα2,3-ST. Moreover, the difference in the magnitude of the change between the Golgi proportion and NB2a cell proportion is consistent with the change being due to an increase in PC rather than a decrease in PE and PS.

One property that both cultured NB2a cell lipids and the lipids from the brain tumors (both ependymoblastoma and CT2A) grown in the mouse flank have in common which is different from rat liver lipids is the presence of a substantial amount of alkylacyl PC (Table 3).[3] In cultured NB2a cell lipids, 15.0% of the phospholipids are alkylacyl PC (14). Because the amount of alkylacyl PC in the liposomes increases as the overall activity of LacCerα2,3-ST decreases (i.e., liver lipids, ependymoblastoma lipids in liver proportion, ependymoblastoma lipids in NB2a cell proportion), it is possible that the amount of alkylacyl PC modulates the total activity of LacCerα2,3-ST although it does not appear to alter the molecular species specificity of the enzyme, which is independently regulated by both the phospholipid class and the molecular species composition of the membrane lipids.

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[3]Ependymoblastoma (and CT2A) tumor lipids contain substantial amounts of alkenylacyl PE while the liver lipids do not, but cultured NB2a cell lipids also do not contain this diradyl form of PE (14).

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