Relationship of membrane phospholipid composition, lactosylceramide molecular species, and the specificity of CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase to the molecular species composition of GM3 ganglioside

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Abstract The ceramide molecular species specificity of rat brain neuron CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase (LacCerα2,3-ST) was determined using 19 molecular species of lactosylceramide incorporated into liposomes prepared with purified rat brain phospholipids. The neuron enzyme displayed a distinct molecular species specificity (which was different than the specificity of liver LacCerα2,3-ST) based on both the long-chain base and the fatty acid composition of the lactosylceramide. Specifically, compared to the liver enzyme, relatively high activities were obtained with d18:1-16:0, d18:1-22:1, and d18:0-18:0 lactosylceramide molecular species. When the lipid composition of the neuron microsomal membranes was altered to resemble that of rat liver Golgi membrane lipids, the activities towards d18:1-16:0, d18:1-22:1, and d18:0-18:0 lactosylceramide molecular species were significantly ($P < 0.01$) reduced and the molecular species specificity of the neuron enzyme resembled that of liver LacCerα2,3-ST. In the reciprocal experiment in which the lipid composition of the rat liver Golgi membranes was altered to resemble neuron microsomal membrane lipids, the molecular species specificity of liver LacCerα2,3-ST was virtually identical to the specificity obtained with the native neuron enzyme. Analysis of the molecular species composition of lactosylceramide and GM3 in rat liver Golgi membranes revealed that the molecular species composition of rat liver Golgi membrane GM3 was precisely what would be expected based on the molecular species specificity of LacCerα2,3-ST and the molecular species composition of lactosylceramide in the Golgi membrane. Based on these results, we conclude that the molecular species specificity of LacCerα2,3-ST determined in our in vitro assay accurately reflects the specificity of the enzyme in vivo and that the specificity of the enzyme is determined by the phospholipid molecular species composition of the Golgi membrane.—**Kadowaki, H., and M. A. Grant.** Relationship of membrane phospholipid composition, lactosylceramide molecular species, and the specificity of CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase to the molecular species composition of GM3 ganglioside. *J. Lipid Res.* 1995. **36:** 1274-1282.

Supplementary key words sialyltransferase • ganglioside • phospholipids • membrane • brain neurons • liver Golgi apparatus

Glycosphingolipids are believed to be located primarily in the outer leaf of the plasma membrane of all mammalian cells. Both the glycosphingolipid class composition and the ceramide molecular species composition of the glycosphingolipids vary among each cell type, and there are changes in both the class and ceramide molecular species composition during normal development (2-4) and tumorogenesis (5-7). The mechanisms responsible for establishing the unique glycosphingolipid class and ceramide molecular species composition of each cell type have not been determined. GM3 ganglioside is the simplest of the acidic glycosphingolipids and is the major glycosphingolipid of most extraneural tissues. GM3 is synthesized by the intrinsic Golgi membrane enzyme CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase (LacCerα2,3-ST) (EC 2.4.99.9) which catalyzes the transfer of *N*-acetylneuraminic acid (NeuAc) from CMP-NeuAc to lactosylceramide (LacCer). LacCerα2,3-ST has recently been purified from rat liver (8) and rat brain (9).

The reaction mechanism of LacCerα2,3-ST is an ordered sequential (Bi Bi) system in which the enzyme first binds LacCer. The affinity of the enzyme-LacCer complex for CMP-NeuAc varies depending on the molecular

Abbreviations: Cer, ceramide; GM3, (NeuAc α2-3)Galβ1-4Glcβ1-1Cer; LacCer, Gal(β1-4)Glc(β1-1)Cer; LacCerα2,3-ST, CMP-*N*-acetylneuraminate:lactosylceramide α2,3-sialyltransferase; NeuAc, *N*-acetylneuraminic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; HPLC, high performance liquid chromatography; RRT, relative retention time. The ceramide molecular species abbreviations suggested by Breimer et al. (1) 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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species of LacCer used as substrate (10). Thus, with in vitro assays, the activity of the enzyme varies depending on the molecular species of LacCer used as substrate. Previous studies (11, 12) demonstrated that the activity of the enzyme could be altered by changing the lipid composition of the Golgi membrane. Specifically, when the lipid composition of rat liver Golgi membrane was altered to resemble that of cultured mouse neuroblastoma NB2a cell microsomes, the characteristic molecular species specificity of liver LacCerα2,3-ST was abolished and the enzyme showed no molecular species specificity for LacCer (11). In cultured NB2a cells, the molecular species composition of LacCer and GM3 (and all the other glycosphingolipids) is the same (13), and when the molecular species specificity of LacCerα2,3-ST of NB2a cells was determined, the enzyme did not display any LacCer molecular species specificity. However, when the lipid composition of NB2a cell microsomal membranes was changed to resemble that of rat liver Golgi membranes, then NB2a cell LacCerα2,3-ST showed a specificity similar to that seen with the rat liver enzyme. When the phosphatidylethanolamine (PE)/phosphatidylcholine (PC) ratio or the amount of phosphatidylserine (PS) in liver Golgi membranes was decreased to that found in NB2a cell microsomes, the activity of liver LacCerα2,3-ST also decreased but the molecular species specificity of the enzyme actually increased (12). Thus, the phospholipid class composition of NB2a cell Golgi membranes is not what is responsible for the lack of the molecular species specificity of NB2a cell LacCerα2,3-ST. The present study was undertaken to further explore the basis for the effect of membrane lipid composition on the molecular species specificity of LacCerα2,3-ST using two tissues (brain neurons and liver) that have similar phospholipid class compositions but different LacCerα2,3-ST molecular species specificities, and to determine whether the molecular species specificity measured in vitro is indicative of the specificity of the enzyme in vivo.

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); analytical and HPLC grade solvents were from Fisher (Medford, MA). LacCer molecular species containing d18:1 or d20:1 long-chain base coupled with either 16:0, 18:0, 20:0, 22:0, 24:0, 20:1, 22:1, or 24:1 fatty acid were chemically synthesized by de- and reacylation of LacCer prepared from bovine brain gangliosides (14). The purity of each LacCer molecular species was over 95% as determined by HPLC.

Animals

Male Sprague-Dawley rats (Taconic Farms Inc., Germantown, NY) weighing 125–150 g were fed standard Purina rat chow ad libitum. Rats were anesthetized and perfused with saline through the left ventricle of the heart to remove residual blood from liver and brain.

Preparation of microsome membranes from brain neurons and Golgi membranes from liver

Brain neurons were isolated as described by Farooq and Norton (15). Neurons were suspended in 1 mM Tris buffer (pH 7.4) and homogenized with a Potter-Elvehjem tissue grinder with Teflon pestle of zero clearance. The concentration of Tris buffer was adjusted to 50 mM and of sucrose to 250 mM, and the homogenate was centrifuged at 10,000 *g* for 10 min. The pellet was washed twice with Tris-sucrose buffer and centrifuged as above. All the supernatants were combined and centrifuged at 105,000 *g* for 60 min. The pellet was suspended in 50 mM Tris buffer (pH 7.4) and sonicated in ice with a microprobe for 2 min (4 × 30 sec). The sample was centrifuged at 105,000 *g* for 60 min and the pellet of neuron microsomal membranes was resuspended in 50 mM Tris buffer (pH 7.4) and resonicated before use. The liver Golgi membrane was prepared as previously described (10) and sonicated before use.

Preparation of liver parenchymal cells

Parenchymal cells were prepared from isolated liver as described by Seglen (16). Cell viability was approximately 90% as determined by trypan blue staining.

Preparation of liposomes

Phospholipids were extracted from perfused rat livers and brain cortex with chloroform–methanol 2:1 and partitioned with saline into two phases (17). The lower phase was separated into neutral lipids and individual phospholipid classes by HPLC (18). Phospholipid classes were quantitated by phosphorus analysis (19). The purified phospholipid classes and cholesterol were dissolved in chloroform, and the brain phospholipids were mixed in the same proportion as they occur in the rat brain neuron microsomal membrane (neuron liposomes) and the liver phospholipids were mixed in the same proportion as they occur in the rat liver Golgi membrane (liver liposomes). The composition of the neuron and liver liposomes is shown in **Table 1.** Liposomes were then prepared with 10 nmol of single molecular species of LacCer and 200 nmol of phospholipids as previously described (20).

TABLE 1. Proportion of lipids in liposomes

Lipids	Liposomes	
	Neuron	Liver
	mol%	
Phosphatidylcholine	45.9 (58.0)	57.8 (62.3)
Phosphatidylethanolamine	19.6 (24.8)	21.2 (22.8)
Phosphatidylinositol	6.7 (8.5)	8.1 (8.7)
Phosphatidylserine	4.5 (5.7)	2.9 (3.1)
Sphingomyelin	2.4 (3.0)	2.8 (3.0)
Cholesterol	20.9	7.2

Lipid composition of neuron liposomes is the same proportion as in the microsomal membranes of rat brain neurons and liver liposomes is the same as in the Golgi membranes of rat liver (11). Mol% of phospholipid classes is presented in parentheses.

LacCerα2,3-ST assay

The activity of LacCerα2,3-ST was determined in the presence of purified nonspecific lipid transfer protein (21) as previously described (20, 22), and the conditions are stated in the legend of Fig. 1.

Analysis of LacCer and GM3 molecular species

Neutral glycosphingolipids in the brain neurons, whole liver, liver parenchymal cells, and liver Golgi membranes were isolated (23) and derivatized with benzoylchloride (24). LacCer was quantitated by HPLC (22). The molecular species of LacCer were determined by HPLC as previously described (25) except that two 3-μm Spherisorb C18 reversed-phase columns (2 × 250 mm, MetaChem Technologies, Redondo Beach, CA) were connected in series and the mobile phase was acetonitrile-2-propanol 8:2 at a flow rate of 0.3 ml/min. The gangliosides were also isolated from these tissues by DEAE-Sephadex column chromatography (26) and derivatized with 2,4-dinitrophenyl hydrochloride (27). GM3 was quantitated by HPLC (13). The molecular species of GM3 were determined by HPLC as described (25) except that two 3-μm Spherisorb C18 reversed-phase columns (2 × 250 mm) were connected in series and the mobile phase was acetonitrile-methanol-water-acetic acid 72:24:4:0.02 at a flow rate of 0.3 ml/min.

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 (28) and conversion of the resulting diglycerides to benzoyl esters (29). Ceramide molecular species of sphingomyelin (SM) were determined as previously described (28) except that the HPLC column was a 3-μm Spherisorb (2 × 250 mm) and the mobile phase was acetonitrile-methanol 1:1. Protein was quantitated by the method of Lowry et al. (30) with bovine serum albumin as standard.

RESULTS

The molecular species specificity of neuron LacCerα2,3-ST (using brain neuron microsomal membranes as the source of enzyme) was determined using various single molecular species of LacCer (i.e., d18:1, d20:1, and d18:0 long-chain bases coupled with 16:0, 18:0, 20:0, 22:0, 24:0, 20:1, 22:1 or 24:1 fatty acid) incorporated into neuron liposomes prepared as described in Experimental Procedures (**Fig. 1,** solid bars). As there was considerable variation in the activity of LacCerα2,3-ST among the neuron microsome preparations, the activity was expressed as the relative activity compared to the activity obtained with d18:1-18:0 LacCer. The activity of neuron LacCerα2,3-ST towards d18:1-18:0 LacCer was 2.5 ± 0.8 nmol/mg microsomal protein per h (mean ± SD of four determinations). With those molecular species of LacCer containing a d18:1 long-chain base and a saturated fatty acid and a d20:1 long-chain base and a monounsaturated fatty acid, the activity decreased nearly linearly with increasing carbon number of the fatty acids. With those molecular species containing a d18:1 long-chain base and a monounsaturated fatty acid, the activity of LacCerα2,3-ST towards 20:1 and 22:1 was similar and was nearly twice that obtained with 24:1. With those molecular species containing a d20:1 long-chain base and a saturated fatty acid, the activities were considerably lower than those of the corresponding fatty acid with d18:1 long-chain base. With those molecular species of LacCer containing d18:0 long-chain base and a saturated fatty acid, the activity towards d18:0-16:0 and d18:0-24:0 was similar to that with d18:1-16:0 and d18:1-24:0, respectively, but the activity towards d18:0-18:0 was twice that of d18:1-18:0.

The activity of the same preparation of neuron LacCerα2,3-ST was then determined using the same 19 molecular species of LacCer incorporated into liver liposomes (Fig. 1, hatched bars). With the liver liposomes the activity towards d18:1-18:0 (2.5 ± 0.3 nmol/mg protein per h, n = 4) was virtually identical to that obtained with neuron liposomes. The molecular species specificity was also similar to that obtained with neuron liposomes except for d18:1-16:0, d18:1-22:0, d18:1-22:1, d20:1-16:0, d20:1-24:1, and d18:0-18:0 molecular species of LacCer. Specifically, the activities towards d18:1-16:0 and d18:1-22:1 decreased by 35% and the activity towards d18:0-18:0 decreased to a half of the activity obtained with neuron liposomes. On the other hand, the activities towards d18:1-22:0, d20:1-16:0, and d20:1-24:1 increased by 80%, 20%, and 100%, respectively, compared to that obtained with the neuron liposomes. Moreover, with the liver liposomes the activity of neuron LacCerα2,3-ST towards both those molecular species with a d18:1 long-chain base and a monounsaturated fatty acid and a d18:0 long-chain base and a saturated fatty acid decreased linearly as the carbon number of the fatty acid increased.

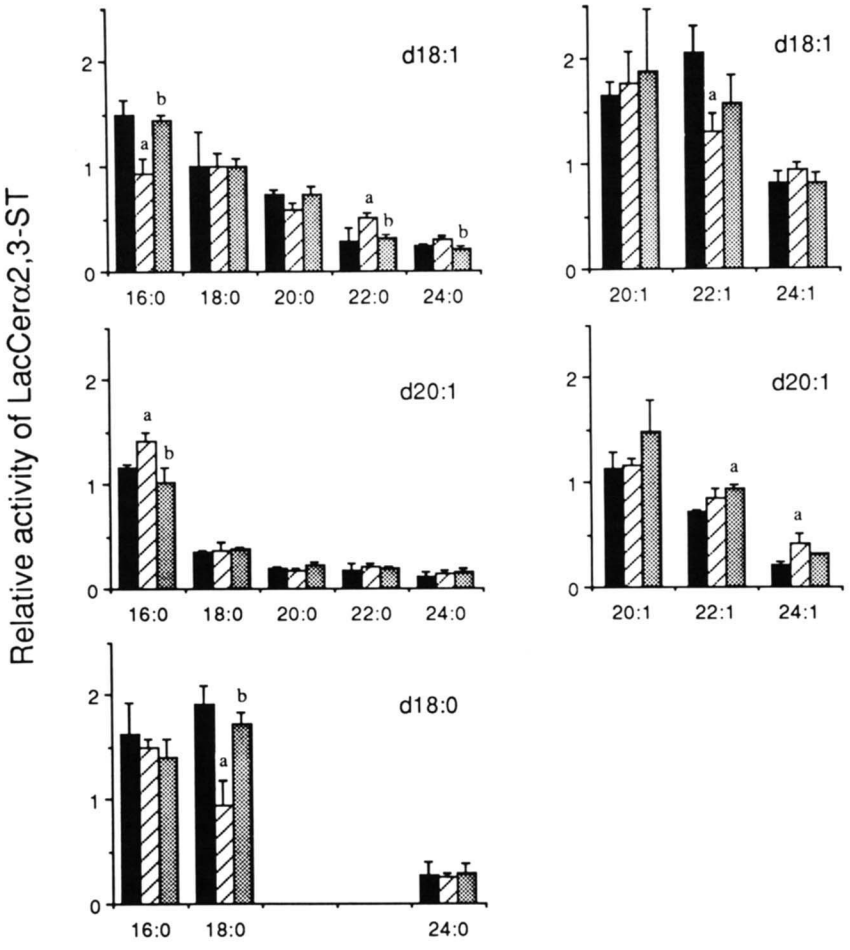

Fig. 1. The effect of liposome lipid composition on the activity of LacCerα2,3-ST. The incubation mixture contained 10 nmol of single molecular species of LacCer incorporated into liposomes (200 nmol of phospholipids), 40–50 μg of purified nonspecific lipid transfer protein, 50 μg of neuron microsome or liver Golgi membrane protein, and 50 nmol of CMP-[^{14}C]NeuAc in a final volume of 50 μl at a final pH of 6.2. The results are the means ± SD of 4 determinations. The activities are expressed as the relative activity compared to the activity obtained with d18:1–18:0 LacCer of each experiment. Solid bars: neuron LacCerα2,3-ST was incubated with neuron liposomes. Hatched bars: neuron LacCerα2,3-ST was incubated with liver liposomes. Dotted bars: liver LacCerα2,3-ST was incubated with neuron liposomes. Long-chain bases are indicated in the upper right-hand corner of each panel and fatty acids are indicated on the abscissa. 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 0.01. a, Statistically different from the activity shown in solid bars; b, from hatched bars.

In a reciprocal experiment, the activity of liver LacCerα2,3-ST was determined using neuron liposomes (Fig. 1, dotted bars). The activity of liver LacCerα2,3-ST towards d18:1–18:0 with neuron liposomes was 5.3 ± 0.4 nmol/mg Golgi protein per h (n = 4) and this value was significantly lower than the activity obtained with liver liposomes; 7.8 ± 1.7 (10). However, the relative activity of liver LacCerα2,3-ST towards the various molecular species of LacCer was similar to that obtained with neuron LacCerα2,3-ST assayed with neuron liposomes. In only one case, d20:1–22:1, was there a significant difference between the activity of liver LacCerα2,3-ST and the neuron enzyme assayed with neuron liposomes.

The phospholipid class compositions of neuron and liver liposomes (Table 1) were similar, but the neuron microsomal membranes contained 3 times the proportion of cholesterol than liver Golgi (20.9 mol% vs. 7.2 mol%). Thus, the changes in the specificity of the enzyme towards the various molecular species of LacCer could be due to the amount of cholesterol in the liposomes. Therefore, the effect of cholesterol (7, 15, 20, 25 mol% of the liposome lipids) on the activity of liver LacCerα2,3-ST using d18:1–18:0 and d18:0–18:0 LacCer molecular species (the two molecular species that showed the least and the greatest alteration of LacCerα2,3-ST activity by changing the membrane lipid composition) was determined using liposomes prepared with liver phospholipids (**Fig. 2**). In this experiment the lowest concentration (7 mol%) of

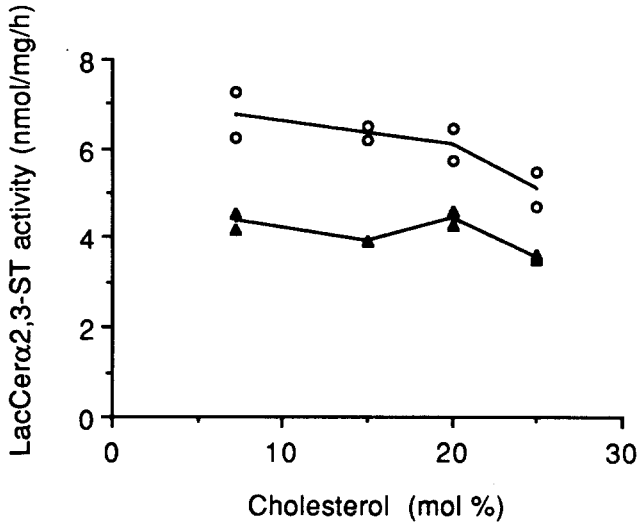

Fig. 2. The effect of cholesterol on the activity of LacCerα2,3-ST. Liver LacCerα2,3-ST was incubated with liver liposomes with various amounts (7, 15, 20, and 25 mol%) of cholesterol. The molecular species tested were; ○, d18:1-18:0 and ▲, d18:0-18:0. The activity was assayed in duplicate.

cholesterol was the same amount as in liver Golgi membranes and 20 mol% was similar to brain neuron microsomes. In all cases, changes in the proportion of cholesterol in the liposomes did not alter the activity of LacCerα2,3-ST. Therefore, the difference in the activity of neuron LacCerα2,3-ST towards d18:0-18:0 between neuron and liver liposomes was not due to the difference in the cholesterol content of the membranes.

Consequently, the acyl chain composition of the phospholipids of the neuron microsomal membranes was determined (**Table 2**) and compared to that of liver Golgi membrane phospholipids (11). Although there were marked variations in the molecular species among the phospholipid classes, the major contribution of the phospholipid molecular species in the liposomes was from PC and PE. Therefore, as seen in the last column of Table 2, the major molecular species of glycerophospholipids in the neuron liposomes were 18:0-22:6 (28 mol%), 16:0-18:1 (23%), and 18:0-20:4 (17%). This is markedly different from liver liposomes in which the major phospholipid molecular species were 18:0-20:4 (22 mol%), 16:0-18:2 (19%), 18:0-18:2 (13%), and 16:0-20:4 (12%) (12). Neuron PC contained ~1% each of the alkenylacyl and alkylacyl forms (the molecular species were not determined) and ~98% as the diacyl form. Neuron PE contained ~38% as the alkenylacyl form, ~3% as the alkylacyl form (molecular species were not determined), and ~60% as the diacyl form. SM represented only 3% of neuron microsomal phospholipids and nearly 70% of SM was d18:1-18:0 (**Table 3**). Thus, the molecular species composition was distinctly different from liver Golgi SM in which d18:1-18:0 was only a minor component (10%) and the major molecular species were d18:1-24:0 (30%), and d18:1-24:1 (17%) (12).

To determine whether the molecular species specificity of LacCerα2,3-ST measured in vitro reflected the molecular species specificity of the enzyme in vivo, the molecular species of LacCer and GM3 in brain neurons, whole liver, liver parenchymal cells, and liver Golgi membranes were compared. Rat brain neurons contained ~2 nmol of LacCer and ~40 nmol of *N*-acetyl GM3/g wet weight. The major molecular species of LacCer and GM3 isolated from neurons was d18:1-18:0 (~40%) (**Fig. 3**). Both LacCer and GM3 contained ~6% of d20:1-18:0 molecular species. There was a clear increase in the proportion of d18:0-18:0 in GM3 compared to LacCer (from 1% to 7%), and neuron LacCerα2,3-ST showed the highest activity towards this molecular species of LacCer (Fig. 1, solid bars). On the other hand, the proportion of d18:1-24:1 in LacCer decreased to one-fourth in GM3 (16% to 4%). Molecular species containing longer chain fatty acids, such as 25:0 (~4%) and 26:0 (~2%), were de-

TABLE 2. Major glycerophospholipid molecular species composition of microsomal membranes prepared from rat brain neurons

	PC	PE				
Molecular species	Diacyl	Alkenyl	Diacyl	PI	PS	Liposomes
			mol%			
16:0-22:6	4.8	15.4	9.9	1.1		6.0
18:1-22:6, 16:0-16:1	1.6	5.7	3.5	0.2		2.1
16:0-20:4, 16:1-18:1	7.8	10.4	2.9	9.6		6.7
18:1-20:4	2.1	5.0	3.0	10.8		2.8
16:0-18:2	1.8		0.4	0.2		1.2
18:1-18:2	0.4					0.2
18:0-22:6	21.9	31.7	34.4	2.6	67.7	27.7
16:0-18:1	35.4	8.1	5.4	0.9	3.2	23.2
18:0-20:4, 18:1-18:1	7.9	23.7	29.6	74.6	13.5	17.3
18:0-18:2	0.8		0.9			0.7
16:0-18:0	7.1		4.3			4.9
18:0-18:1	8.4		5.7		14.1	7.2

The notation used for glycerophospholipid molecular species is not intended to indicate the position of the fatty acid on the glycerol. The distribution of the molecular species in liposomes (last column) was calculated from the concentration of each glycerophospholipid classes in the neuron microsomal membranes (Table 1).

TABLE 3. Major sphingomyelin molecular species of microsomal membranes isolated from rat brain neurons

Molecular species	mol %
d18:1-16:0	11.1
d18:1-17:0	0.6
d18:1-18:0	67.9
d18:1-20:0	10.4
d18:1-22:0	2.9
d18:1-23:0	0.6
d18:1-24:0	2.2
d18:1-24:1	3.5
d18:1-24:2	0.8

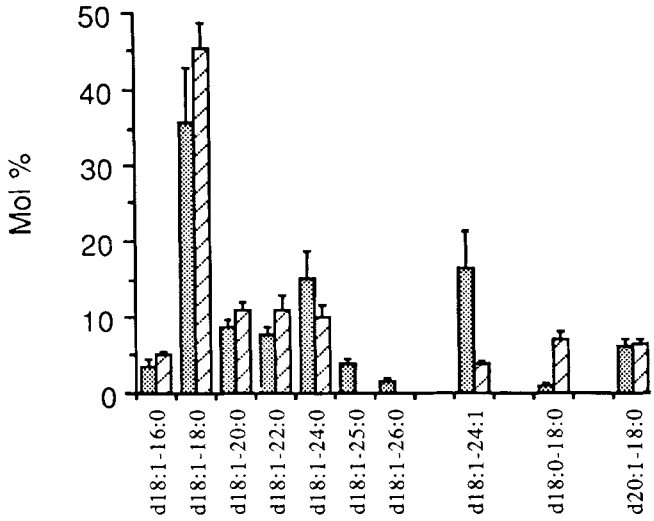

Fig. 3. The distribution of molecular species in LacCer and GM3 isolated from rat brain neurons. Dotted bars, LacCer; hatched bars, GM3. The results are means ± SD of 3 separate neuron preparations.

tected in LacCer but not in GM3.

Perfused whole rat liver contained ~0.08 nmol of LacCer and ~16 nmol of *N*-acetyl GM3/g wet weight. The major long-chain base in liver glycosphingolipids was d18:1. The molecular species analysis of whole liver LacCer and GM3 showed that the major molecular species were d18:1-24:0 (27%), d18:1-16:0 (20%), d18:1-22:0 (15%), and d18:1-24:1 (15%) and there were only small differences in the distribution of molecular species between LacCer and GM3 (**Fig. 4A**). As liver contains many cell types, the parenchymal cells were isolated from perfused livers and the molecular species of LacCer and GM3 were determined. Liver parenchymal cells contained ~0.06 nmol of LacCer and ~12 nmol of *N*-acetyl-GM3 in the parenchymal cells isolated from 1 g of liver. The distribution of the molecular species was not different between LacCer and GM3, and was also similar to the distribution obtained with whole liver (Fig. 4B). Golgi membranes isolated from 1 g of liver contained ~0.005 nmol of LacCer and ~5 nmol of *N*-acetyl-GM3. When the molecular species of LacCer and GM3 isolated from liver Golgi membranes were analyzed, the molecular species distribution in LacCer was similar to the distribution of molecular species observed in the LacCer of whole liver and parenchymal cells (Fig. 4C). However, the molecular species distribution of Golgi membrane GM3 was clearly different from the distribution of molecular species of LacCer and GM3 in the whole liver and parenchymal cells. Specifically, the proportion of d18:1-16:0, d18:1-18:0 and d18:1-22:1 in GM3 was twice that in LacCer, and the proportion of d18:1-22:0, d18:1-23:0, d18:1-24:0 and d18:1-25:0 in GM3 was significantly less than in LacCer. Although the proportion of d18:1-26:0 in LacCer was low (~1%), this molecular species was not detected in GM3.

DISCUSSION

In the assay system used in the present study, the lipophilic substrate, LacCer, is transferred from carrier liposomes to the membrane where the enzyme is located by nonspecific lipid transfer protein (22). We have previously

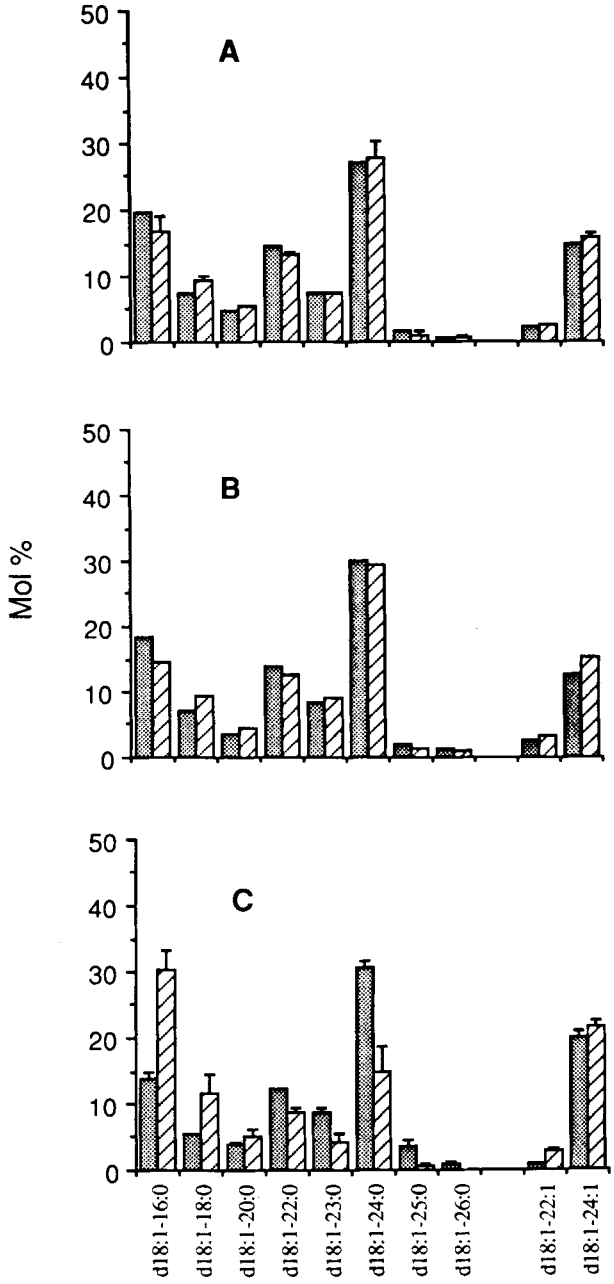

Fig. 4. The distribution of molecular species in LacCer and GM3 isolated from rat liver. Dotted bars, LacCer; hatched bars, GM3. (A) Whole liver; LacCer (n = 2 with less than 5% variations) and GM3 (means ± SD, n = 3). (B) Liver parenchymal cells (n = 2 with less than 7% variations). (C) Liver Golgi membranes; LacCer (means ± SD, n = 3), each determination was performed with Golgi membranes isolated from 5-6 livers; and GM3 (n = 3), each determination was performed with Golgi membranes isolated from 1-3 livers.

shown that nonspecific lipid transfer protein has no molecular species specificity for LacCer (12, 20). Nonspecific lipid transfer protein also facilitates the exchange of phospholipids and cholesterol between the membranes and liposomes (21, 31, 32). As the liposomes in the incubation mixture contain approximately 10 times as much phospholipid as the neuron microsomal or liver Golgi membranes, after incubation with nonspecific lipid transfer protein the lipid composition of those membranes largely reflects the composition of the liposomes. As was shown in Fig. 1, when neuron LacCerα2,3-ST was incubated with liver liposomes, the molecular species specificity of the neuron enzyme was changed and was virtually identical to that of native liver enzyme (10). Likewise, when liver Golgi membranes were changed to resemble the lipid composition of neuron microsomes by incubating them with neuron liposomes, the molecular species specificity of the liver enzyme was virtually identical to that of the native neuron enzyme. A similar phenomenon was observed previously using rat liver Golgi and cultured mouse neuroblastoma cell LacCerα2,3-ST (11). Thus, in this assay system the molecular species specificity of LacCerα2,3-ST resembled the molecular species specificity of the cells from which the liposome lipids were derived rather than the cells (liver Golgi, brain neuron microsomes, or NB2a cell microsomes) from which the enzyme was derived even when the enzyme and lipids are from different species. This strongly suggests that the LacCer molecular species specificity of LacCerα2,3-ST observed in any cell is determined primarily by the lipid composition of the membrane in which the enzyme is located.

There are only small differences in the phospholipid class composition of rat brain neuron microsomal membranes and rat liver Golgi membranes, and it is therefore unlikely that differences in the molecular species specificity of liver and neuron LacCerα2,3-ST are due to differences in the phospholipid class composition of the two membrane preparations. In a previous study (12), changes in the phospholipid class composition of the membrane, particularly PC, PE, and PS, were shown to alter the molecular species specificity of liver LacCerα2,3-ST, but relatively large changes in the phospholipid class composition resulted in only relatively small changes in the specificity of the enzyme, and the direction of the changes observed in the previous study cannot explain the differences in the molecular species specificity of liver and neuron LacCerα2,3-ST observed in the present study. As shown in Fig. 2, differences in the proportion of cholesterol in the two membranes also cannot explain the difference in the molecular species specificity between liver and neuron LacCerα2,3-ST. Thus, it is likely that the differences in the molecular species specificity of LacCerα2,3-ST are due to differences in the molecular species composition of the membrane phospholipids in those tissues. Previous studies (11, 12) with neuroblastoma cells also suggested that the molecular species composition of neuroblastoma cell phospholipids was responsible for the lack of LacCerα2,3-ST molecular species specificity observed in those tumors. In that study, however, it was not possible to rule out the possibility that the distribution of the diradyl forms of PC and PE could also be involved, but, based on the results of the previous and the present studies taken together, that possibility now appears unlikely because there is no consistent variation in the molecular species specificity of LacCerα2,3-ST with the proportion of alkylacyl or alkenylacyl lipids in the enzyme assay system.

Although it can be shown that the molecular species specificity of LacCerα2,3-ST can be altered by changes in the lipid composition of the membrane where the enzyme is located in an in vitro assay, the question remains as to whether a similar specificity is observed in vivo, where other regulatory factors could be more influential. (For instance, we have previously shown (10) that in the in vitro assay, the extent of the molecular species specificity of LacCerα2,3-ST depends on the concentration of CMP-NeuAc.) When the molecular species compositions of LacCer and GM3 were determined in the rat liver Golgi membrane, there were indeed differences in the molecular species composition of the substrate (LacCer) and the product (GM3) of the LacCerα2,3-ST reaction. When the ratio of GM3 to LacCer was calculated for each molecular species and that ratio was plotted against the

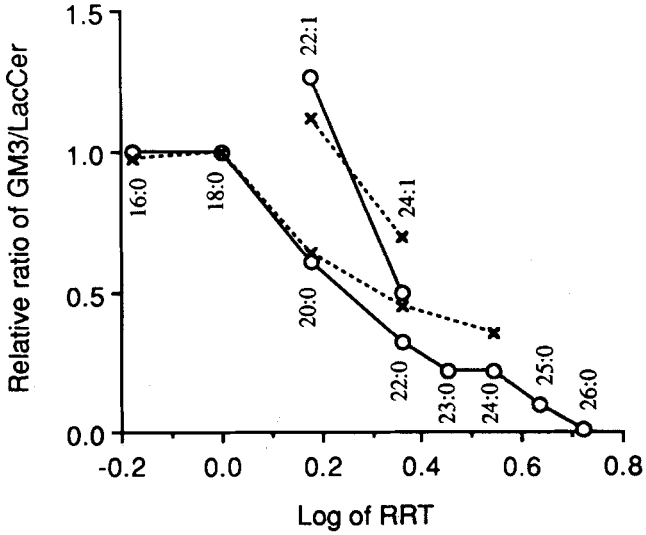

Fig. 5. The relative ratio of GM3/LacCer molecular species proportion in the rat liver Golgi membranes. The values were obtained from Fig. 4C. The relative ratio was calculated by dividing the ratio of GM3/LacCer in each molecular species by the ratio obtained with d18:1–18:0 molecular species, and plotted in the response to log of relative retention time (RRT) of each molecular species (solid lines). RRT is calculated by dividing the absolute RT of each molecular species of LacCer by the RT of d18:1–18:0 LacCer (20). The relative activity of liver LacCerα2,3-ST obtained in the previous study (10) is presented to compare the profile of the specificity (dashed lines). Long-chain base of all molecular species is d18:1 and fatty acids are indicated in the figure.

effective carbon number of that molecular species [log of relative retention time (RRT)] (**Fig. 5,** solid lines), virtually the identical pattern was obtained as when the activity of liver LacCerα2,3-ST for each molecular species determined in the in vitro assay was plotted against the log of RRT of the respective molecular species of LacCer (dashed lines) (10). Thus, at least for rat liver, the specificity of LacCerα2,3-ST observed in our in vitro assay accurately reflects the molecular species specificity of LacCerα2,3-ST in vivo. A similar comparison of the molecular species composition of LacCer and GM3 in the neuron Golgi was not possible because the Golgi apparatus preparation from isolated neurons is only ~30% Golgi apparatus (33), and the yield is very low and GM3 is only a very minor ganglioside in neurons. However, a comparison was made between the neuron LacCer and GM3, and there were differences between the molecular species composition of LacCer and GM3, but in general those differences do not reflect the molecular species specificity of neuron LacCerα2,3-ST. This is not unexpected because it is likely that, as is the case with liver parenchymal cells, the molecular species composition of Golgi GM3 and whole cell GM3 is different. However, the activity of neuron LacCerα2,3-ST towards d18:0–18:0 is very high, and this is reflected in the difference between neuron LacCer and GM3 in which the proportion of d18:0–18:0 in GM3 is ~7 times higher than in LacCer.

Based on these results, we conclude that the molecular species specificity of LacCerα2,3-ST determined in our in vitro assay reflects the specificity of the enzyme in vivo and that the specificity of the enzyme is determined by the phospholipid molecular species composition of the Golgi membrane.

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