Kidney lipids in galactosylceramide synthase-deficient mice: absence of galactosylsulfatide and compensatory increase in more polar sulfoglycolipids[1]

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Abstract UDP-galactose:ceramide galactosyltransferase (CGT) catalyzes the final step in the synthesis of galactosylceramide (GalCer). It has previously been shown that CGT-deficient mice do not synthesize GalCer and its sulfated derivative GalCer I^3-sulfate (galactosylsulfatide, SM4s) but form myelin containing glucosylceramide (GlcCer) and sphingomyelin with 2-hydroxy fatty acids. Because relatively high concentrations of GalCer and SM4s are present also in mammalian kidney, we analyzed the composition of lipids in the kidney of *Cgt*$^{-/-}$ and, as a control, *Cgt*$^{+/-}$ and wild-type mice. The homozygous mutant mice lacked GalCer, galabiaosylceramide (Ga_2Cer), and SM4s. Yet, they did not show any major morphological or functional defects in the kidney. A slight increase in GlcCer containing 4-hydroxysphinganine was evident among neutral glycolipids. Intriguingly, more polar sulfoglycolipids, that is, lactosylceramide II^3-sulfate (SM3) and gangliotetraosylceramide II^3,IV^3-*bis*-sulfate (SB1a), were expressed at 2 to 3 times the normal levels in *Cgt*$^{-/-}$ mice, indicating upregulation of biosynthesis of SB1a from GlcCer via SM3. Given that SM4s is a major polar glycolipid constituting renal tubular membrane, the increase in SM3 and SB1a in the mice deficient in CGT and thus SM4s appears to be a compensatory process, which could partly restore kidney function in the knockout mice.—Tadano-Aritomi, K., T. Hikita, H. Fujimoto, K. Suzuki, K. Motegi, and I. Ishizuka. **Kidney lipids in galactosylceramide synthase-deficient mice: absence of galactosylsulfatide and compensatory increase in more polar sulfoglycolipids.** ***J. Lipid Res.*** **2000.** 41: **1237–1243.**

Supplementary key words UDP-galactose:ceramide galactosyltransferase • gene targeting • glycolipids • galactosylceramide • sulfatides • sulfolipids • sulfoglycolipids • kidney • liquid-SIMS • TLC blotting

Galactosylceramide (GalCer) and its sulfated derivative, GalCer sulfate (galactosylsulfatide, SM4s), are present most abundantly in the brain of vertebrates, comprising almost one-third of the lipid mass of myelin. [Note: Abbreviations for sulfoglycolipids follow the modifications of the Svennerholm system (1), and the designation of the other glycolipids follows the IUPAC-IUB recommendations (2).] Knockout mice with a disrupted UDP-galactose:ceramide galactosyltransferase (CGT) gene have been generated by gene targeting (3–5). The homozygous mutant mice, which are incapable of synthesizing either GalCer or SM4s, form myelin containing elevated amount of glucosylceramide (GlcCer) and sphingomyelin with 2-hydroxy fatty acids. Nevertheless, these mice display a variety of deficits in myelin structure, function, and stability, indicating that both GalCer and SM4s are indispensable components of myelin (4, 6). GalCer and/or SM4s are present in glandular epithelial tissues including kidney and the islet of Langerhans (7), where sulfoglycolipids are believed to be essential components of surface membrane as the amphiphilic donor of negative charges (8). Although the function of GalCer and SM4s in these tissues is not clear as yet, CGT-deficient mice should provide useful keys to delineate the roles of sulfolipids. Here, we analyze the lipids in the kidney of *Cgt*$^{-/-}$ mice in comparison with those of *Cgt*$^{+/-}$ and wild-type mice and investigate the effect of elimination of GalCer and SM4s on renal function.

Abbreviations: Cer, ceramide; DMB, 1,2-diamino-4,5-(methylenedioxy)benzene; HexCer, monohexosylceramide; Hex_2Cer, dihexosylceramide; GalCer, galactosylceramide; Ga_2Cer, galabiaosylceramide, Galα4GalCer; LacCer, lactosylceramide, Galβ4GlcCer; Gb_3Cer, globotriaosylceramide, Galα4Galβ4GlcCer; Gb_4Cer, globotetraosylceramide, GalNAcβ3Galα4Galβ4GlcCer; GM3 (NeuAc), II^3-α-NeuAc-LacCer; GM3(NeuGc), II^3-α-NeuGc-LacCer; SM4s, galactosylceramide sulfate (galactosylsulfatide), GalCer I^3-sulfate; SM3, lactosylceramide sulfate (lactosylsulfatide), LacCer II^3-sulfate; SB1a, Gg_4Cer II^3,IV^3-bis-sulfate, (HSO_3-3)Galβ3GalNAcβ4(HSO_3-3) Galβ4GlcCer; HSO_3-Chol, cholesterol 3-sulfate; HFA, 2-hydroxy fatty acid; d18:1, 4-sphingenine; t18:0, 4-hydroxysphinganine; PAS, periodic acid Schiff; TLC, thin-layer chromatography; LSIMS, liquid secondary ion mass spectrometry.

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MATERIALS AND METHODS

Mice

Mice heterozygous ($Cgt^{+/-}$) and homozygous ($Cgt^{-/-}$) for the disrupted *Cgt* gene were generated as described previously (4). In the mutant allele, the *Cgt* gene was inactivated by insertion of the neomycin resistance gene into exon 2, which encodes the N-terminal half of the CGT enzyme. The mice heterozygous for the disrupted *Cgt* gene were originally supplied by B. Popko (University of North Carolina School of Medicine, Chapel Hill, NC) and maintained in the Mitsubishi Kasei Institute of Life Sciences (Tokyo, Japan) by backcrossing to C57BL/6N. The mice used for experiments were generated by interbreeding of heterozygotes. For genotyping of mice, DNA isolated from tail biopsy was digested with *Bam*HI, and hybridized with pCR550 (kindly supplied by B. Popko) containing a 595-nucleotide (nt) fragment of *Cgt* exon 2 as a probe for Southern blot analyses. A 15-kb band was detected for the wild type, and DNA from the $Cgt^{-/-}$ mice contained 8- and 9-kb bands. Heterozygotes had all three bands.

Kidney function tests were done in the laboratory of one of the authors (K.M.) by adapting routine clinical analysis procedures to the mouse, using serum or urine samples from $Cgt^{-/-}$ (n = 4), $Cgt^{+/-}$ (n = 7), and wild-type (n = 5) mice. Detailed procedures will be provided on request.

Histology

Kidneys were dissected and fixed in Bouin's solution overnight. After dehydration, tissues were embedded in paraffin wax and 7-μm sections were stained for the periodic acid–Schiff (PAS) reaction followed by hematoxylin staining.

Lipid extraction and analysis

Pooled kidneys (2–8 g) from 7- to 12-week-old mice of each genotype and sex were extracted with 19 volumes of chloroform–methanol 2:1 (v/v) and with 10 volumes of chloroform–methanol–water 60:120:9 (v/v/v) successively (9). The pooled extracts (the total lipid extract) were chromatographed on a DEAE-Sephadex A-25 column. After washing out neutral lipids, acidic lipids were eluted sequentially by a concave gradient of ammonium acetate (10). A part of the pooled fractions was treated with 0.4 M methanolic NaOH to destroy glycerophospholipids.

Acidic lipids were further separated on silica gel 60 high-performance thin-layer chromatography (TLC) plates (E. Merck, Darmstadt, Germany) in chloroform–methanol–water 60:40:9 (v/v/v) containing 0.2% $CaCl_2$ or 3.5 M NH_4OH, or chloroform–methanol–acetone–acetic acid–water 7:2:4:2:1 (v/v/v/v/v). The total lipid extract and the neutral glycolipid fraction were analyzed by two-dimensional TLC, using the solvent systems chloroform–methanol–water 60:40:9 or 60:35:8 (v/v/v) containing 0.2% $CaCl_2$ (first direction) and chloroform–methanol–acetone–acetic acid–water 7:2:4:2:1 (v/v/v/v/v) (second direction). The solvent for the second direction was replaced by chloroform–methanol–$(CH_3O)_3B$ 50:20:1 (v/v/v) for the separation of GlcCer from GalCer (11), and by 2-propanol–15 M NH_4OH–methyl acetate–water 5:2:1:3 (v/v/v/v) for the separation of lactosylceramide (LacCer) from galabiaosylceramide (Ga_2Cer) (12). Ga_2Cer (16:0 + 24:0/d18:1) from porcine pancreas (13) was kindly provided by K. Nakamura (Kitasato University School of Medicine, Kitasato, Japan). The bands were visualized with orcinol (hexose-containing lipids) (14), Azure A (sulfolipids) (11), resorcinol (gangliosides) (15), or cupric acetate–phosphoric acid (phospholipids and other lipids) (16). Lipids on the plates were determined by densitometry (CS-9000; Shimadzu, Kyoto, Japan) by comparison with known amounts of authentic standards.

Identification of lipids was performed by negative-ion liquid secondary ion mass spectrometry (LSIMS) on a Concept IH mass spectrometer (Shimadzu/Kratos, Kyoto, Japan) (17). Each lipid developed by one- or two-dimensional TLC was transferred to a polyvinylidene difluoride membrane (Clear Blot Membrane-P; ATTO, Tokyo, Japan) by TLC blotting, and the band on the membrane was excised and placed on a mass spectrometer probe tip with triethanolamine as the matrix (18).

The concentrations of lipid-bound sulfate, sialic acids, and phosphorus were determined with an aliquot of the neutral lipid fraction or the pooled fraction from DEAE-Sephadex. For sulfate, the sample was hydrolyzed in 1 M HCl, and SO_4^{2-} released was determined by ion chromatography (Tadano-Aritomi et al., unpublished observations) (19). Ganglioside sialic acids were determined as their 1,2-diamino-4,5-(methylenedioxy)benzene (DMB) derivatives by high-performance liquid chromatography (HPLC) (20). Phospholipid phosphorus was measured by the malachite green method (21).

RESULTS

Characteristics of CGT-deficient mice

The homozygous mutant mice (7 to 12 weeks of age) exhibited a characteristic phenotype, with a body weight two-thirds that of wild-type and $Cgt^{+/-}$ littermates (4). The kidney weight of $Cgt^{-/-}$ mice was approximately two-thirds that of wild-type and heterozygous littermates, simply reflecting the lower body weight of $Cgt^{-/-}$ mice. Other than the smaller size, no evidence of abnormality was recognized in the kidney of $Cgt^{-/-}$ mice (data not shown). To examine the renal function of $Cgt^{-/-}$ mice, several parameters including blood urea nitrogen (BUN), creatinine, Na^+, K^+, Cl^-, urinary osmolality, and β-*N*-acetyl-D-glucosaminidase (NAG) excretion were measured in serum or urine samples. The data (mean ± SD) are as follows: BUN (mg/dL), 33 ± 5 ($Cgt^{-/-}$), 28 ± 5 ($Cgt^{+/-}$), 30 ± 2 (wild type); creatinine (mg/dL), 1.1 ± 0.2 ($Cgt^{-/-}$), 1.0 ± 0.1 ($Cgt^{+/-}$), 1.0 ± 0.2 (wild type); Na^+ (mEq/L), 152 ± 3 ($Cgt^{-/-}$), 149 ± 3 ($Cgt^{+/-}$), 149 ± 4 (wild type); K^+ (mEq/L), 5.3 ± 1.7 ($Cgt^{-/-}$), 5.1 ± 1.3 ($Cgt^{+/-}$), 4.9 ± 0.3 (wild type); Cl^- (mEq/L), 118 ± 1 ($Cgt^{-/-}$), 117 ± 2 ($Cgt^{+/-}$), 116 ± 2 (wild type); urinary osmolality (mOsm/kg), 2,137 ± 28 ($Cgt^{-/-}$), 1,600 ± 438 ($Cgt^{+/-}$), 1,922 ± 237 (wild type); NAG (U/L), 64 ± 12 ($Cgt^{-/-}$), 47 ± 10 ($Cgt^{+/-}$), 61 ± 12 (wild type). All of these data were within the normal range and no significant differences were observed among $Cgt^{-/-}$, $Cgt^{+/-}$, and wild-type mice.

GalCer and Ga_2Cer are absent from neutral lipids

As shown in **Fig. 1**, neutral glycolipids consisted of monohexosylceramide (HexCer), dihexosylceramide (Hex_2Cer), globotriaosylceramide (Gb_3Cer), and globotetraosylceramide (Gb_4Cer) as well as more polar glycolipids. Consistent with previous studies (22, 23), the profile of the male differed conspicuously from that of the female. The bands corresponding to Hex_2Cer and Gb_3Cer were significantly reduced not only in the kidney of the female mice of three genotypes but also in the $Cgt^{-/-}$ male mice. To differentiate LacCer and Ga_2Cer, the neutral lipid fractions were analyzed by two-dimensional TLC with 2-propanol–15 M NH_4OH–methyl acetate–water (12) as the second

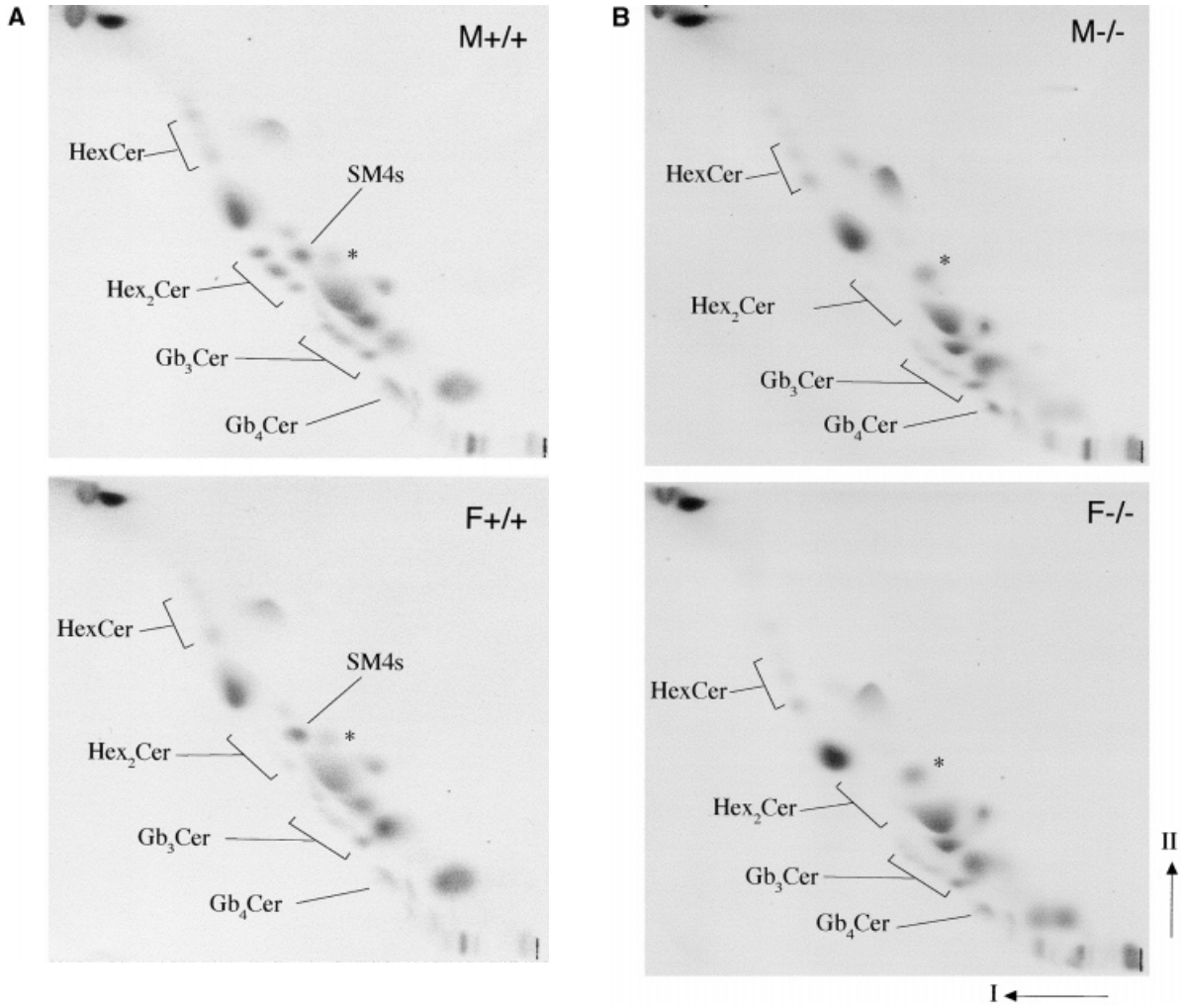

Fig. 1. (A and B) Two-dimensional TLC of a total lipid extract from the kidney of wild-type and $Cgt^{-/-}$ mice of both sexes. An aliquot of the total lipid extract corresponding to 2 mg of kidney was separated on a TLC plate with chloroform–methanol–water 60:35:8 (v/v/v) containing 0.2% $CaCl_2$ (first direction) and chloroform–methanol–acetone–acetic acid–water 7:2:4:2:1 (v/v/v/v) (second direction). Glycolipid bands were visualized with the orcinol reagent. Asterisks (*) indicate unidentified constituents that moved close to SM4s but appeared brownish with orcinol reagent. M, Male; F, female.

developing solvent (data not shown). The three bands, which migrated faster than LacCer but similar to Ga_2Cer (24), were detected only in the kidney of the male wild-type and $Cgt^{+/-}$ mice. Together with their LSIMS spectra, these bands were identified as Ga_2Cer with major fatty acid/sphingoid of 24:0/d18:1, 16:0/d18:1, and 16h:0/d18:1, respectively (**Table 1**). As expected, Ga_2Cer was also absent in the kidney of $Cgt^{-/-}$ mice. LacCer could be detected neither in the kidney of $Cgt^{-/-}$ mice nor in those of $Cgt^{+/-}$ and wild-type littermates (22) with the sensitivity of the analytical procedure used in this study.

HexCer represented a minor component among glycolipids in the kidney of six mice groups (three genotypes in both sexes) (Fig. 1). By two-dimensional TLC with the $(CH_3O)_3B$-containing solvent followed by negative-ion LSIMS, four faint bands were identified as GlcCer(24h:0/d18:1), GlcCer(24:0/t18:0), GlcCer(24h:0/t18:0), and GalCer(24h:0/d18:1) (Table 1). In the kidney of $Cgt^{-/-}$ mice, the band corresponding to GalCer was absent. Instead, increases in GlcCer bands containing 4-hydroxysphinganine (t18:0) were characteristic, although they represented only minor components among neutral glycolipids in the kidney of $Cgt^{-/-}$ mice.

Lack of SM4s produces a conspicuous increase in more polar sulfoglycolipids

The bands of individual sulfolipid was identified by negative-ion LSIMS (Table 1). The major sulfolipid, SM4s, comprises almost 75% (**Table 2**) of the sulfolipids in the kidney of wild-type mice with three minor sulfolipids, that is, LacCer II^3-sulfate (SM3), Gg_4Cer II^3,IV^3-bis-sulfate (SB1a) (25), and cholesterol 3-sulfate (HSO_3-Chol) (8). As expected, TLC analysis demonstrated a lack of SM4s in the kidney of $Cgt^{-/-}$ mice (Fig. 1). Instead, a substantial increase in SB1a with a small increase of SM3, respectively, was the remarkable feature of the kidney of $Cgt^{-/-}$ mice as compared with those of wild-type and $Cgt^{+/-}$ littermates (**Fig. 2**). In the kidney of $Cgt^{-/-}$ mice, the amounts

TABLE 1. Negative-ion LSIMS analysis of major lipids

Compound	*m/z*	Ion	Structure
Neutral glycolipids			
GlcCer	826	$[M-H]^-$	24h:0/d18:1
	828	$[M-H]^-$	24:0/t18:0
	844	$[M-H]^-$	24h:0/t18:0
GalCer[a]	826	$[M-H]^-$	24h:0/d18:1
Ga_2Cer[a]	972	$[M-H]^-$	24:0/d18:1
	988	$[M-H]^-$	24h:0/d18:1
	860	$[M-H]^-$	16:0/d18:1
	876	$[M-H]^-$	16h:0/d18:1
Sulfolipids and gangliosides			
SM4s[a]	906	$[M-H]^-$	24h:0/d18:1
	778	$[M-H]^-$	16:0/d18:1
SM3	1070	$[M-H]^-$	24:0/t18:0
	1042	$[M-H]^-$	22:0/t18:0
SB1a	1491	$[M+Na-2H]^-$	22:0/d18:1
	1519	$[M+Na-2H]^-$	24:0/d18:1
	1537	$[M+Na-2H]^-$	24:0/t18:0
HSO_3-Chol	465	$[M-H]^-$	
GM3(NeuAc)	1263	$[M-H]^-$	24:0/d18:1
GM3(NeuGc)	1279	$[M-H]^-$	24:0/d18:1
Phospholipids			
PI	885	$[M-H]^-$	18:0/20:4
PS	810	$[M-H]^-$	18:0/20:4
CL	1447	$[M-H]^-$	$(18:2)_4$
SM	799	$[M-15]^-$	24:0/d18:1

The DEAE-Sephadex fractions containing each lipid were pooled and an aliquot was separated by one- or two-dimensional TLC. Each band on the plate was analyzed with a triethanolamine matrix after blotting to polyvinylidene difluoride membranes.

[a] Not detected in the kidney of $Cgt^{-/-}$ mice.

TABLE 2. Concentrations of sulfolipids in the kidney

	Concentration (nmol SO_4^{2-}/g wet tissue)			
	SM4s	SM3	SB1a	HSO_3-Chol
Male				
+/+	275	22	20	44
+/−	290	27	18	42
−/−	ND	42	65	42
Female				
+/+	296	24	21	44
+/−	240	22	20	58
−/−	ND	44	69	58

Abbreviation: ND, not detected.

Each pooled fraction of DEAE-Sephadex containing SM4s + SM3, HSO_3-Chol, or SB1a was hydrolyzed in 1 M HCl at 100°C for 3.5 h. After partition in the Folch system, SO_4^{2-} in the upper phase was determined by ion chromatography. The ratios of SM3 to SM4s in the kidneys of wild-type and $Cgt^{+/-}$ mice were determined by TLC-densitometry after staining with Azure A. Data are expressed as the means of duplicate experiments.

of SM3 and SB1a, determined by ion chromatography, were approximately 2- and 3-fold, respectively, the level of wild-type or $Cgt^{+/-}$ mice, while the level of HSO_3-Chol remained unchanged (Table 2). In contrast to the homozygote sciatic nerve (26), no compensatory appearance of glucosylsulfatide (GlcCer I^3-sulfate) (11) was noted in the kidney of $Cgt^{-/-}$ mice.

Gangliosides and phospholipids are unchanged

TLC profiles of ganglioside fractions were essentially similar among the six mouse groups (data not shown).

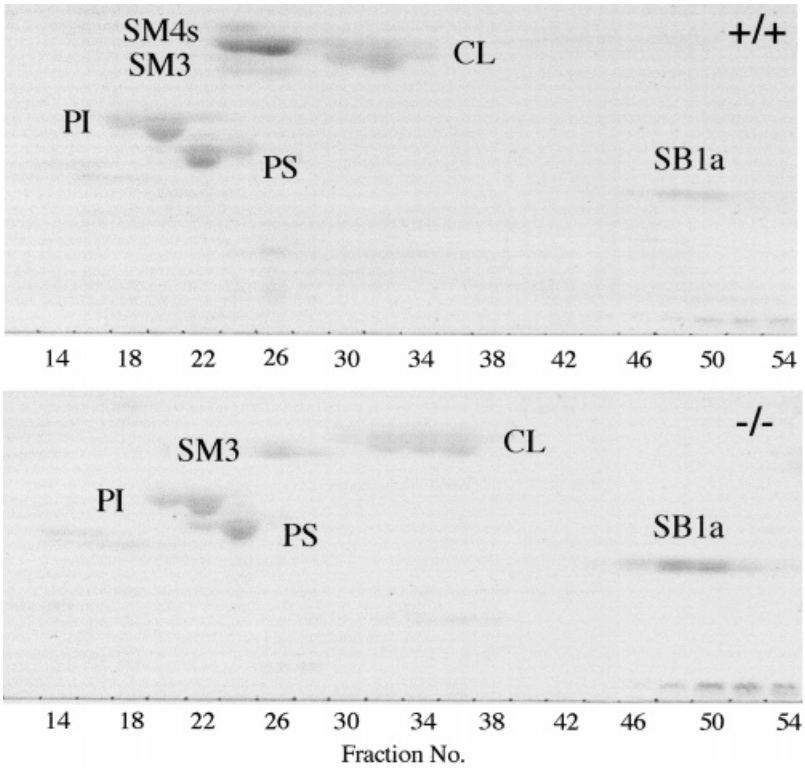

Fig. 2. Elution profiles of acidic lipids from the kidney of female wild-type (top) and $Cgt^{-/-}$ (bottom) mice. Acidic lipids were eluted from a DEAE-Sephadex A-25 column by a concave gradient of ammonium acetate, and samples from every two tubes were applied on the plate. The plate was developed in chloroform–methanol–water 60:40:9 (v/v/v) containing 0.2% $CaCl_2$ and stained with the orcinol reagent.

Major gangliosides identified by LSIMS were GM3(NeuAc) and GM3(NeuGc) (Table 1), which comprised 40% (10.2 nmol/g) and 25% (6.2 nmol/g), respectively, of the total ganglioside sialic acids (26 nmol/g) in the kidney of the male wild-type mice. HPLC analyses of NeuAc and NeuGc in mono-, di-, and trisialosyl fractions from DEAE-Sephadex showed no significant differences in their concentrations among the six groups (data not shown). These finding on kidney gangliosides was consistent with the normal brain ganglioside composition in *Cgt*$^{-/-}$ mice (27).

The composition of major acidic phospholipids including phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL), as well as sphingomyelin (SM), in *Cgt*$^{-/-}$ kidney was compared with those in wild-type and *Cgt*$^{+/-}$ littermates. No changes in the composition and concentration of these phospholipids could be observed among the six groups (data not shown). In the kidney of male wild-type mice, concentrations (micromoles of PO_4 per g wet tissue) of PS, PI, CL, and SM were 3.8, 2.5, 6.3, and 3.0, respectively. Major molecular species were identified as PS(18:0/20:4), PI(18:0/20:4), $CL(18:2)_4$, and SM(24:0/d18:1) by negative-ion LSIMS (Table 1). Unlike the brain of *Cgt*$^{-/-}$ mice (4), SM containing 2-hydroxy fatty acids (HFA-SM) could not be detected in the kidney.

DISCUSSION

Transcripts of the *Cgt* gene were clearly detected in the normal kidney (28) and testis (29, 30) in addition to the cerebrum and cerebellum (data not shown), suggesting that the loss of this enzyme activity may affect the function of these tissues and consequently contribute to the phenotype of the mutants. With this hypothesis in mind, we examined changes in the lipid composition of the kidney of CGT-deficient mice.

HexCer from the kidney of wild-type mice consisted of four species, of which one was identified as GalCer and the others as GlcCer. It has been reported that both GlcCer containing 2-hydroxy fatty acids (HFA-GlcCer) and HFA-SM were expressed in compensation for GalCer in the brain of CGT-deficient mice (4). In the kidney of *Cgt*$^{-/-}$ mice, GalCer was absent, as expected. In contrast, two bands of GlcCer containing t18:0 sphingosine, which are the major molecular species of HexCer in rat kidney (11), were substantially increased. No differences were seen in the level and species of SM; HFA-SM could be detected neither in *Cgt*$^{-/-}$ mice nor in wild-type and *Cgt*$^{+/-}$ littermates. It has been expected that in the absence of CGT activity, the lack of GalCer and Ga_2Cer could stimulate the synthesis of GlcCer as well as LacCer in compensation. Unexpectedly, LacCer could hardly be detected in the kidney of six groups, suggesting its prompt conversion to SM3 and GM3.

Staining with monoclonal antibodies showed that sulfoglycolipids are distributed on the lumenal (apical) cell surface of renal tubules (7, 31). The enrichment of sulfolipids in osmoregulatory organs including kidney and intestine has suggested that sulfolipids play important roles as the ion barrier at the cell membrane (8). The present study confirms our hypothesis that the sum of sulfoglycolipids is more concentrated in the kidney of smaller animals (8, 32), suggesting that the glycolipid-bound sulfate may participate more actively in the kidney of smaller animals such as mice. However, CGT-deficient mice lacking SM4s showed neither morphological defects in the kidney nor abnormality in parameters responsible for renal function. Although these findings do not completely exclude the possibility that GalCer and/or SM4s may be dispensable for normal kidney function, there are other possibilities that are consistent with their functional importance.

First, in place of SM4s, *Cgt*$^{-/-}$ mice still express more polar sulfoglycolipids, that is, SM3 and SB1a, in the kidney. Moreover, both sulfoglycolipids are expressed at higher levels in *Cgt*$^{-/-}$ mice than in wild-type and *Cgt*$^{+/-}$ littermates, indicating that the biosynthetic pathway of SB1a from GlcCer via LacCer and SM3 is stimulated in the kidney of *Cgt*$^{-/-}$ mice (**Fig. 3**), similar to the changes in SM4s and SM3 in MDCK cells under osmotic stresses (33). In contrast, no increase in the levels of HSO_3-Chol as well as gangliosides was observed. Because of the formidable compensatory capacity of the kidney cells to the osmotic environments ranging between 0 and 1200 mOsm, it is possible that the increment of SB1a and SM3 can, at least partially, compensate for the lack of SM4s and allow keeping the normal function of the kidney in *Cgt*$^{-/-}$ mice.

Second, it could not be ruled out that acidic phospholipids may partly compensate for the SM4s deficiency.

Third, subtle abnormalities undetectable by routine examinations under normal environment, might be present in the kidney of CGT-deficient mice. Experimental manipulations, such as osmotic stress by dehydration, may uncover such a borderline functional state of the SM4s-deficient renal tubules.

Brigande, Platt, and Seyfried (34) reported enhanced synthesis of GalCer-related lipids SM4s and GM4 in the mouse embryo treated with an inhibitor of ceramide glu-

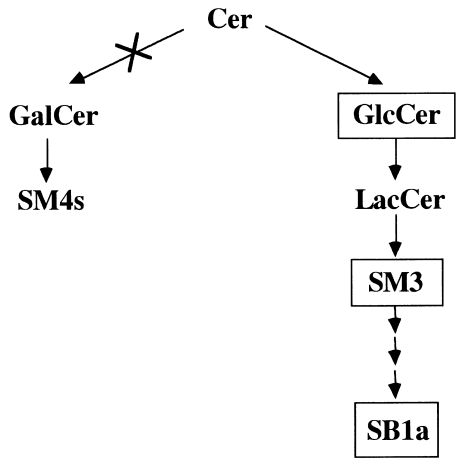

Fig. 3. Proposed pathway of sulfoglycolipids biosynthesis. In *Cgt*$^{-/-}$ mice, the crossed-out step is blocked. The boxed items represent the glycolipid, the concentration of which is increased in the kidney of *Cgt*$^{-/-}$ mice.

cosyltransferase. They suggested that the increases in GalCer-related lipids may be an adaptive response to prevent the accumulation of potentially harmful upstream metabolites, for example, ceramide. We find these authors' argument too teleological. Our view is that the increased synthesis of the GalCer series of lipids under glucosyltransferase-inhibited conditions, as well as that of polar sulfoglycolipids, in our *Cgt* knockout mice occurs simply because more acceptor molecules become available due to the metabolic block and thus can go to other synthetic pathways that have a higher K_m for the acceptor. Under both conditions, ceramidase is present in excess and therefore accumulation of ceramide to a harmful concentration can easily be prevented by degrading it even without increasing the synthetic side reactions. In fact, quantitative estimates indicated clearly that the increase in GlcCer in the kidney of $Cgt^{-/-}$ mice is minor and most of the excess ceramide is being degraded by ceramidase.

The CGT-deficient mouse should allow further analysis of the specific role of GalCer and its derivatives in the kidney. Because the CGT-deficient mouse generates neither SM4s nor its precursor, GalCer, precise dissection of the effect of the precursor and its sulfated end product is difficult as yet. GalCer sulfotransferase has been cloned (35, 36) and we can soon expect a mutant mouse lacking the capacity to generate sulfated glycolipids. Comparison of the CGT knockout mouse with the expected sulfotransferase knockout mouse could answer the vital question concerning whether both GalCer and SM4s or only SM4s is important for function. The data presented in this article should provide the basis for such a comparison.

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