Fabry disease is an X-linked inherited loss of α-galactosidase A (α-Gal A). Affected patients experience complications that include neuropathy, renal failure, and cardiovascular disease. Although the genetic and biochemical basis of this sphingolipidosis is well studied, the basis for the vascular disease remains poorly understood. In an attempt to create a suitable in vitro model of this disease, conditions for the growth of primary cultures of aortic endothelial cells from wild-type and α-Gal A −/− mice were established. The cultured cells demonstrated CD-31 expression by flow cytometry and LDL binding by immunofluorescence. The glycolipid expression patterns were compared between wild-type and α-Gal A null cells. Importantly, cells from α-Gal A −/− mice but not α-Gal A +/+ mice expressed high levels of the globo-series glycosphingolipid globotriaosylceramide (Gb3). The age-dependent elevation in Gb3 was measured. By 4 mo of age, α-Gal A −/− mouse aortic endothelial cells achieved their peak Gb3 levels. The ability to lower Gb3 levels pharmacologically was assessed next. The glucosylceramide synthase inhibitor ethylenedioxyphenyl-P4 significantly lowered but did not eliminate Gb3 levels by 96 h of treatment. Gb3 synthesis was completely blocked as measured by [14C]galactose labeling. Recombinant α-Gal A more significantly lowered Gb3 levels by 48 h but had a more limited effect on de novo synthesis. Together, both agents eliminated detectable Gb3. In summary, primary cultures of aortic endothelial cells from Fabry mice retain the phenotype of elevated globo-series glycosphingolipids. These cells provide a useful model for comparing pharmacologic agents used for glycolipid reduction.


Materials and Methods

Reagents

mAb MC631 (anti-Gb4 and Gb5) was obtained as a hybridoma supernatant from the Developmental Studies Hybridoma Bank (Iowa, IA) (9). Biotinylated anti-mouse IgG and IgM, avidin-linked alkaline phosphatase, and alkaline phosphatase substrate were acquired from Vector Laboratories (Temecula, CA). n-[1-14C]galactose was from Amersham Biosciences (Buckinghamshire, UK). Acetylated LDL fluorescently labeled with Dil (Dil-Ac-LDL) was purchased from Biomedical Technologies Inc. (Stoughton, MA). Anti-CD31-PE and anti-CD11b-PE were obtained from BD Biosciences (Bedford, MA). Fabrazyme was provided by Genzyme Corporation (Cambridge, MA). n-[1-14C]EtDO-P4 was synthesized and purified as described previously (10).
Animals
α-Gal A–deficient mice (Gla−/0), provided by National Institutes of Health (Bethesda, MD), were bred on normal diet and maintained under standard pathogen-free conditions at the University of Michigan. Wild-type C57BL/6j mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

Isolation of Mouse Aortic Endothelial Cells
Mice at ages of 3 to 4 mo were used for most experiments. The mouse thoracic aortas were removed from the proximal portion to the middle portion. The aortas were immediately washed in ice-cold RPMI medium that contained 100 units/ml penicillin and 100 μg/ml streptomycin to remove blood and gently dissected from fat and connective tissues under a dissecting microscope. The aortas then were cut into approximately 2-mm rings that were placed carefully onto collagen-coated (type I) culture wells. Mouse aortic endothelial cells (MAEC) first were grown in complete plating RPMI medium that consisted of 20% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 0.5% nonessential amino acids, 0.1 mg/ml heparin, and 0.05 mg/ml ECGS (BD Biosciences, MA). At culture day 5, when endothelial cells had detached and grown onto the surface of the culture dishes, the aortic rings were discarded and MAEC were cultured continually in complete RPMI medium until confluent (usually 10 d) as passage 0 (P0) cells.

MAEC Cultures and Treatments
MAEC at P0 were released from collagen by incubation with 0.25% collagenase (type I) at 37°C for 10 min. Collected P0 MAEC were washed with RPMI medium and replated onto 1% gelatin-coated culture dishes as passage 1 cells. Confluent passage 1 and 2 MAEC were released with trypsin-EDTA treatment and maintained in complete RPMI medium that contained 15% FBS. Unless otherwise indicated, MAEC at passage 3, cultured in 10% FBS-RPMI medium, were used for all experiments and chemical or enzyme replacement treatments. α1-EIDO-P4 was dissolved in 100% DMSO. The stock solution was diluted further with RPMI medium before addition to cell cultures. α-Gal A (Fabrazyme) was freshly reconstituted with RPMI medium and then diluted further with complete RPMI medium before use.

Flow Cytometry
Cultured MAEC were released by trypsin-EDTA treatment. The released cells were washed gently two times by adding PBS buffer supplemented with 0.5% BSA and 2 mM EDTA. The cell pellets were resuspended into 100 μl of PBS-0.5% BSA-2 mM EDTA buffer and stained with anti-CD31-PE (1 μg/10^6 cells) at 4°C for 15 min or stained with anti-CD11b-PE as a negative control for leukocytes and dendritic cells. After labeled MAEC were washed in PBS-0.5% BSA-2 mM EDTA buffer two times, they were identified by flow cytometry.

Uptake of Dil-Ac-LDL
MAEC at passage 3 or control NIH 3T3 cells were cultured in two-chamber slides until 95% confluent (>36 h after Trypsin-EDTA treatment). Dil-Ac-LDL was added to cultured cells at a final concentration of 20 μg/ml. Cells then were incubated with Dil-Ac-LDL for at least 6 h at 37°C. The incubations then were stopped by removing the media. Cells then were washed by probe-free RPMI medium twice before fluorescence microscopy. Alternatively, the cells were washed with ice-cold PBS and fixed in 3% formaldehyde/probe-free RPMI medium for 20 min at 4°C. The gasket was removed carefully, and slides were rinsed by dipping in distilled water for 5 s at room temperature. The liquid drained slides were mounted with a drop of mounting solution that contained 90% glycerol and 10% PBS and sealed with Premount solution (Sigma, St. Louis, MO). Dil-Ac-LDL labeling was visualized via fluorescence microscopy.

Glycosphingolipid Mass Analysis
Whole cellular lipids were extracted from cultured cells as described previously (11). After determination of total lipid phosphate, crude lipids then were subjected to both base and acid hydrolysis. Equal amounts of whole cellular lipids (400 to 500 nmol of total phospholipids) from a variety of samples were dissolved in 2 ml of chloroform and were incubated with 1 ml of base-hydrolysis solution that contained 0.21 N NaOH in 100% methanol at 37°C for 1 h. The reactions were stopped by the addition of 0.8 ml of 0.25 N HCl. The aqueous and organic phases were separated by centrifugation at 1200 × g for 5 min. The lower organic phases that contained neutral glycosphingolipids were extracted carefully and mixed with 4 ml of methanol and incubated with 1.6 ml of acid-hydrolysis solution that consisted of 0.05 N HCl and 25 mM HgCl₂ at 37°C for 15 min. The reaction was terminated by the addition of 2 ml of chloroform and 1.6 ml of water. After centrifugation at 1200 × g for 5 min, the upper aqueous layer was discarded and the lower organic layer was washed once with 2 ml of methanol and 1.6 ml of 30 mM EDTA and twice with 2 ml of methanol and 1.6 ml of double-distilled water. The glycosphingolipids in chloroform solution were dried under a stream of nitrogen gas and separated by HPTLC. The tissue lipids were extracted from Fabry mouse hearts as described previously (6). For separation of neutral glycosphingolipids, TLC plates first were running in 100 ml of chloroform/methanol (98/2, vol/vol) for 30 min and then developed in a solvent system that consisted of chloroform/methanol/water/acetic acid/NH₄OH (64/31/3/2/0.5, vol/vol/vol/vol/vol) for 50 min. The glycosphingolipids were detected by charring with 8% cupric sulfate in methanol/water/H₃PO₄ (8/60/32/8, wt/vol/vol/vol). The levels of glucosylceramide, lactosylceramide, Gb₃, and Gb4 were quantified by densitometric scanning with NIH Image 1.62 software and compared with authentic standards (Matreya, Inc., Pleasant Gap, PA) run in parallel on the same plates.

Radiolabeling of Glycosphingolipids
Wild-type or α-Gal A−/−/0 MAEC (passage 3) were grown to 80 to 85% confluence in 150-mm culture dishes. The whole cellular lipids were radiolabeled by the addition of [d-1-14C]galactose (0.2 μCi/ml, specific activity; 57.0 mCi/mmol) for 20 h before harvesting. The whole cellular lipids were extracted and quantified by total phosphate assay. Equal amounts of whole cellular lipids (400 nmol total phosphate) were loaded directly on TLC plates or subjected to base and acid hydrolysis. Radiolabeled glycosphingolipids were identified by autoradiography after separation by HPTLC.

Statistical Analyses
All data are expressed as mean ± SEM. Each experiment presented was repeated on three separate MAEC cultures. The data were evaluated using t test. A value of P < 0.05 was considered statistically significant.

Results
MAEC Culture and Characterization
The culture and the growth of MAEC, in vitro, were dependent on the genotypes of mice used in this study. Although the MAEC isolated from both Gla−/0 and Gla+/0 mice grew free of aortic rings in plating medium after 2 d culture (Figure 1), by culture day 10, the number of MAEC that were harvested from...
10 age-matched Gla+/0 mice was 2.0 to 2.3 $\times 10^6$, whereas the MAEC yield from the same number of Gla−/0 mice was 1.5 to 1.6 $\times 10^6$. Under identical culture conditions, a longer culture time (2 to 3 d) was required for Gla−/0 MAEC to reach 90% confluence when compared with the culture and growth of Gla+/0 MAEC, even though the number of cells seeded was always the same. Because endothelial cells express CD31, antibodies to CD31 have been used as a specific and highly sensitive tool to identify vascular endothelial cells.

The MAEC that were grown in culture were characterized with anti-CD31 antibody (12). The presence of anti–CD31-PE–positive cells was determined by flow cytometric analysis. More than 90% of the cultured cells were labeled by anti-CD31-PE (Figure 2C) when compared with background, eliminating smooth muscle cells as a potential contaminant (Figure 2A). CD11b, a marker expressed by leukocytes (13), served as a negative control to eliminate the potential presence of dendritic cells and nonspecific PE labeling (Figure 2B). Approximately 5 to 6% of the total cell population was composed of dead cells (Figure 2A). The endothelial phenotype was also verified by the binding and incorporation of Dil-tagged Ac-LDL (14). The fluorescence intensities of cultured Gla−/0 and Gla+/0 MAEC were comparably high, although not every cell was labeled (Figure 3, A and B). By contrast, there was no active uptake of Dil-Ac-LDL by cultured NIH 3T3 cells (Figure 3, C and D).

**Accumulation of Gb3 in α-Gal A−Deficient MAEC**

The expression of globo-series glycosphingolipid (GSL) in cultured Gla+/0 and Gla−/0 MAEC first was investigated by HPTLC immunostaining with shigatoxin (for Gb3) and mAb
MC631 (for Gb4 and Gb5). On the basis of the mobility and staining of authentic globo-GSL standards in HPTLC plates, the immunobinding of Gb3 was observed in cultured Gla\textsubscript{H11002}/0 MAEC. The Gb3 expression in Gla\textsubscript{H11001}/0 MAEC was not detected by HPTLC immunostaining. Gb4 was expressed in both Gla\textsubscript{H11001}/0 and Gla\textsubscript{H11002}/0 MAEC; however, no Gb5 was detected (data not shown).

The expression of Gb3 in Gla\textsubscript{H11001}/0 or Gla\textsubscript{H11002}/0 MAEC was also measured by the incorporation of \([^{14}\text{C}]\)galactose into cell lipids that were extracted from both Gla\textsubscript{H11001}/0 and Gla\textsubscript{H11002}/0 MAEC. With whole cellular lipids, radiolabeled Gb3 accumulated in cultured Gla\textsubscript{H11001}/0 MAEC (Figure 4A). Using this more sensitive measure of Gb3, a trace amount of \([^{14}\text{C}]\)-labeled Gb3 was detected in cultured Gla\textsubscript{H11001}/0 MAEC. For eliminating possible nonspecific radiolabeling, the cell lipids were purified further by base and acid hydrolysis to remove the more abundant glycerolipids. The resultant neutral GSL from Gla\textsubscript{H11001}/0 and Gla\textsubscript{H11002}/0 MAEC were compared by HPTLC charring with 8% cupric sulfate in 8% phosphoric acid (Figure 4B). In cultured Gla\textsubscript{H11001}/0 MAEC, no Gb3 accumulation was detected in the neutral GSL fraction. A significant accumulation of Gb3 was observed in Fabry MAEC. There were no statistical differences in Gb4 levels from the MAEC between Fabry and wild-type mice. The age-dependent accumulations of Gb3 in MAEC and in mouse heart tissue were examined and compared (Figure 5). By HPTLC analysis, in Gla\textsubscript{H11001}/0 mouse hearts, there were no detectable Gb3 (data not shown), whereas, in Gla\textsubscript{H11002}/0 mice, there were significant accumulations of Gb3. In Gla\textsubscript{H11002}/0 mice, the levels of Gb3 in hearts were gradually increased linearly up to 12 mo of age. Gla\textsubscript{H11002}/0 MAEC Gb3 displayed a different accumulation pattern. The level of Gb3 in Gla\textsubscript{H11002}/0 MAEC from 1-mo-old mice was readily detectable. The accumulation of Gb3 in Gla\textsubscript{H11002}/0 mice rapidly plateaued by 3 to 4 mo of age.
Clearance of Accumulated Gb3 by Substrate Inhibition and Recombinant Protein Treatment

Removal of excess Gb3 in Gla−/0 MAEC was studied by two different pharmacologic strategies. Substrate deprivation was performed by treatment of cultured Gla−/0 MAEC with dt-EtDO-P4, an active and specific inhibitor of glucosylceramide-based glycosphingolipids (11). This compound specifically blocks glucosylceramide formation, the base cerebroside in Gb3. Since the inhibitory activity of dt-EtDO-P4 was significantly reduced by binding to serum contained in culture medium, the embedded MAEC were subcultured in medium containing gradually reduced serum concentrations from 20% in P0 cells to 10% in P3 cells. In this study dt-EtDO-P4 was added to the culture medium at a relatively higher dose to overcome the serum effect (15).

Depletion of Gb3 in Gla−/0 MAEC by 1/H9262 M dt-EtDO-P4 was time dependent (Figure 6). With increasing incubation time, a 50% depletion of Gb3 was observed after 96 h of treatment. Increasing the concentrations of dt-EtDO-P4 to 2 or 3/H9262 M did not further decrease Gb3 levels. However, increased doses of dt-EtDO-P4 did significantly deplete Gb4 levels in Gla−/0 MAEC, a downstream globo-GSL (data not shown). Thus, a second pathway for Gb3 accumulation in cultured MAEC was possible, viz. the catabolism of Gb4. To evaluate this possibility, we examined the incorporation of [14C]galactose into the glycosphingolipids of cultured cells. After incubation with dt-EtDO-P4 for 48 h, the synthesis of new Gb3 in Gla−/0 MAEC was completely blocked, consistent with the requirement for de novo glucosylceramide formation for Gb3 synthesis. Some incorporation of radiolabel into Gb4 was observed, consistent with the conversion of Gb3 to Gb4 (Figure 7). These observations are consistent with the interpretation that loss of Gb3 mass in Gla−/0 MAEC is due primarily to conversion to Gb4.

Enzymatic depletion of Gb3 in Gla−/0 MAEC was carried out using a recombinant human α-Gal A (Fabrazyme). The reduction of Gb3 in Gla−/0 MAEC that were treated with α-Gal A was concentration and time dependent. The longer the incubation time, the lower the dose of enzyme required. Using 20 μg/ml, by 48 h, >97% of the Gb3 in cultured Gla−/0 MAEC was depleted by recombinant human α-Gal A (Figure 8). However, when [14C]galactose was used as a sensitive probe to track synthesis of new globo-series GSL, newly synthesized Gb3 was detected in Gla−/0 MAEC even in the presence of recombinant human α-Gal A for 48 h (Figure 9). This synthesis was present even at high concentrations of enzyme (60 μg/ml).
The combination of dt-EtDO-P4 and recombinant human α-H9251-Gal A on the removal of Gb3 was assessed using a single and low dose for a shorter period of incubation (Figure 10). Cultured Gla/H11002 MAEC were exposed to 0.5 μM dt-EtDO-P4 or recombinant human α-Gal A (1 μg/ml) or a mixture of 0.5 μM dt-EtDO-P4 plus 1 μg/ml recombinant human α-Gal A for 24 h. dt-EtDO-P4 and recombinant human α-Gal A alone were used as controls. Treatments were terminated by the replacement of media, and the cells were cultured continually in media without dt-EtDO-P4 and recombinant human α-Gal A for another 3 d. When lipids were immediately extracted from Gla/H11002 MAEC that had been treated with 0.5 μM dt-EtDO-P4 for 24 h, only 8% of the Gb3 was depleted (data not shown). However, with extended culture time, the effect of low-dose dt-EtDO-P4 on the depletion of Gb3 was enhanced, increasing from 8 to 40%. This effect of low-dose dt-EtDO-P4 was observed even though the inhibitor had been removed and fresh medium was replaced daily. The long-term effect of dt-EtDO-P4 on Gb3 deletion indicated that there was slow turnover of the small molecule inhibitor once incorporated by the cultured cells. A synergistic effect of dt-EtDO-P4 and recombinant human α-Gal A on the loss of excess Gb3 was readily observed. Complete Gb3 clearance was achieved by combined treatment and maintained for at least 72 h in cultured Gla/H11002 MAEC.

**Discussion**

The relationship between Gb3 accumulation in the setting of α-Gal A deficiency and the clinical manifestations of heart, kidney, skin, nerve, and brain disease is not well understood. Thus, understanding the pathophysiology of Fabry disease, like other lysosomal storage disorders, remains a challenge. This study represents an attempt to develop a new tool for such studies. We report that primary cultures of MAEC retain the Fabry phenotype as measured by persistently high Gb3 levels. The elevation in Gb3 persists through early cell passages and is maximal in cells that are obtained from mice of 3 to 4 mo of age. The age-dependent kinetics of Gb3 accumulation in the cultured endothelial cells should be interpreted cautiously, how-
ever, because the independent effects of serum concentration were not studied in detail.

Our study was performed with endothelial cells derived from the aorta. The cultured cells retain an endothelial phenotype based on CD31 expression and LDL binding. Although not all of the cultured cells were labeled with these markers, these findings suggested that a marked enrichment of endothelial cells had been achieved. A more homogeneous culture would have necessitated a positive selection step, presumably at the expense of cell yield. Because other vascular beds, including those of skin and brain, have been implicated in the pathology of Fabry disease, it will be important to determine whether endothelial cells derived from these tissues display similar properties.

Premature vascular disease, particularly stroke, is a devastating complication of Fabry disease. Historically, this has been attributed to microvascular disease, most specifically in association with the endothelial accumulation of glycolipids associated with the ischemic pathology of nephropathy and neuropathy. Historically, little attention has been paid to the large-vessel biology and pathology of the patient with Fabry disease.

In clinical studies in which α-Gal A deficiency is associated with abnormalities of cerebral blood flow (20), the dysregulation of nitric oxide (NO) has been postulated to play a role. However, this has been difficult to document fully in either humans or the intact mouse. In support of a NO-based mechanism for the macrovascular disease, our group recently reported two abnormalities in the α-Gal A null mouse. These abnormalities include a highly robust carotid artery thrombosis in response to oxidant release by rose Bengal (7) and accelerated atherosclerosis in mice bred on an ApoE−/− background (8). In the first model, Gla−/− mice thrombosed their carotid arteries in as little as 15 min after oxidant release. Arterial thrombosis in C57BL/6J mice occurred after 35 min. This susceptibility to thrombosis was age dependent and gender independent. In the second model, the atherosclerosis was most apparent by 45 wk of age, when the lesion area had more than doubled over the aorta and other large arteries. In this study, inducible NO synthase but not endothelial NO synthase expression was significantly elevated in the vessels of the α-Gal A null mice.

For these reasons, endothelial cells were chosen as an initial cell type for the in vitro studies described in this article. Our study was performed on endothelial cells derived from aortic rings, although the eventual comparison of these findings to endothelial cells derived from other vascular beds in which pathology has been well established is important and will be pursued. The establishment of an in vitro model for the study of Gla−/− endothelial cells beginning with aortic endothelium should aid in the study of these models of large-vessel vasculopathy.

Glycosphingolipids can be classified by charge (neutral or acidic) and the presence of chemical modifications, such as sulfation (sulfatides) or sialylation (gangliosides). Glycosphingolipids are also classified as families. Five families are typically expressed in mammalian tissues and include gala, globo (type 4 chain), lacto (type 1 chain), neolacto (type 2 chain), and ganglio. Most glycosphingolipids share a lactosylceramide core and differ in the sequence and anomeric linkage of the third (globo, α1–4Gal; ganglio, β1–4GalNAc) or fourth (lacto, neolacto) oligosaccharide.

Gb3 is a globo-series glycosphingolipid. The globo-series glycosphingolipids are among the most widely distributed glycosphingolipids in human and vertebrate tissues. They serve as antigens on blood cells, epithelia, and other tissues. Globo-series glycosphingolipids (Figure 11) are also oncofetal antigens and are expressed at the early cleavage stage in embryos and are markers of embryonic mesoderm, extra-embryonic visceral endoderm, and visceral yolk sac cells (21,22). In early embryogenesis, globose (Gb4) and two other related stage-specific antigens (Gal-Gb4 and LKE) play significant roles in preimplantation development (23). Their absence may be lethal (24,25). Functionally, the expression and role of globo-glycosphingolipids in early mammalian development may mirror those ascribed to the arthro-series glycosphingolipids in drosophila. Brainiac is a β3-N-acetylglucosaminyltransferase related to globose synthase (β3GalT3) (26,27). The arthro-series glycosphingolipids in drosophila play critical roles in oogene-
Figure 11. Pathways for Gb3 metabolism. Structures, common abbreviations, and primary enzymes for globo-series glycosphingolipids are shown. Synthesis of globo-series glycosphingolipids is dependent on the action of multiple, distinct glycosyltransferases beginning with the formation of glucosylceramide. All globo-glycosphingolipids are derived from a common precursor, glucosylceramide. All globo-series GSL are important markers for breast, prostate, testicular, and renal cell carcinoma, in which they have been implicated in cell adhesion, proliferation, and signaling. Many globo-series GSL are also important host factors in the pathophysiology of several infectious diseases (36). The colonization of epithelial cells by Streptococcus suis, Escherichia coli, and Pseudomonas aeruginosa is through the recognition of globo-series GSL (37–39). Gb4 serves as the receptor for parvovirus B19, and Gb3 is required for the CD4/CXCR4-dependent fusion of HIV (40,41). Gb3 is also the receptor for the shiga toxins, Stx 1 and 2, that are the etiologic agents for Shigella dysentery, E. coli food poisoning, and hemolytic uremic syndrome (42,43).

Several therapeutic approaches to the treatment of Fabry disease have been proposed. These include gene therapy, bone marrow transplantation, enzyme replacement therapy, and the use of small molecule inhibitors of glycosyltransferase. Currently, only enzyme replacement has achieved routine clinical use. Small molecule inhibitors of glycolipid syntheses have targeted glucosylceramide synthase, the first step in glycosphingolipid synthesis. The 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP)-based synthase inhibitors have been the most extensively studied group of these compounds (44). A pharmacophore required for enzyme inhibition was previously identified. The core structure has been amenable to substitutions that have led to the identification of homologues with increasingly greater activity and specificity. More recently, by the use of rational drug design, phenyl substitutions with ethylenedioxy- or 4-hydroxy groups resulted in the discovery of homologues with low nanomolar IC50 (10). These inhibitors block glucosylceramide formation without significant changes in ceramide levels.

The accumulation of Gb3 and strategies designed to lower tissue Gb3 levels need to be viewed in the context of glycosphingolipid metabolism in general and globo-series glycosphingolipid metabolism in particular (Figure 11). An intervention that lowers Gb3 by catabolism to lactosylceramide or by inhibition of an upstream glycosyltransferase will predictably have secondary effects on the levels of other glycolipids. Because these related sphingolipids may have independent biologic activity, it seems important to evaluate these secondary changes. In this study, we compared the effects of the glucosylceramide synthase inhibitor pd-EtDO-P4 with recombinant a-Gal A. Although both agents significantly lowered cellular Gb3 mass, recombinant a-Gal A was superior in its effect. However, radiolabeling experiments indicated that de novo Gb3 synthesis occurred even in the presence of the glycosidase, consistent with persistent activity of the synthetic enzymes.

These experiments also demonstrated that de novo Gb4 formation occurred in the presence of pd-EtDO-P4. Thus, increased Gb4 synthesis in the absence of an intact catabolic pathway may be one means whereby a-Gal A-deficient tissues balance Gb3 content. This finding may also explain a previous in vivo observation in the Fabry mouse. When Gla−/− mice were treated with pd-EtDO-P4, Gb3 levels in kidney decreased to levels below those observed in untreated mice (6). This finding raised the possibility that the Fabry mice had an alternative pathway for lowering Gb3. The formation of more complex glycolipids such as Gb4 may be one such pathway.

References


