Distribution of α-Galactosidase A in Normal Human Kidney and Renal Accumulation and Distribution of Recombinant α-Galactosidase A in Fabry Mice

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Deficiency of lysosomal α-galactosidase A (α-Gal A) in Fabry disease results in cellular accumulation of globotriaosylceramide (G3), often leading to end-stage renal failure. G3 accumulates in endothelial, glomerular, and tubular cells. Replacement therapy with recombinant α-Gal A to some extent reduces cellular accumulation of G3 in the kidney. This study shows high lysosomal expression of α-Gal A in all tubular segments and interstitial cells of normal human kidney. However, glomeruli and endothelial cells did not express the enzyme to any significant extent. Recombinant enzyme was taken up by rat yolk sac cells in a receptor-associated protein–inhibitive manner, and surface plasmon resonance experiments revealed binding to and endothelial cells did not express the enzyme to any significant extent. Recombinant enzyme was taken up by rat yolk sac cells in a receptor-associated protein–inhibitive manner, and surface plasmon resonance experiments revealed binding to and endothelial cells. In conclusion, intravenously administered enzyme is taken up by interstitial cells, is to some extent filtered in glomeruli, and is taken up by podocytes and reabsorbed by receptor-mediated endocytosis in proximal tubule cells, directly indicating a potential beneficial effect of enzyme replacement therapy for these cells.

Fabry-Anderson disease is an X-linked disease that results from mutations of the gene that encodes the lysosomal enzyme α-galactosidase A (α-Gal A) (1). The reduced amounts of functional enzyme result in accumulation of globotriaosylceramide (G3) in a variety of cells of various organs, including brain, heart, and kidney. In addition, endothelial cells throughout the vasculature accumulate G3. The disease generally affects male individuals earlier and more severely than female individuals (1,2), and in classically affected male individuals, it often results in cerebrovascular manifestations, heart failure, renal failure, and early death. However, many heterozygous female individuals may be affected similarly to hemizygous male individuals (3). Recently established enzyme replacement therapy (ERT) results in significant clearance of the endothelial deposits in kidney, heart, and skin but also to some extent in the renal glomerular and tubular deposits (4). In a preclinical study on α-Gal A gene–deleted mice, it was demonstrated that approximately 1% of the injected recombinant enzyme could be found in the kidney 1 h after infusion (5); however, the renal distribution of infused recombinant enzyme has not been described.

To our knowledge, the normal renal distribution of α-Gal A has not been described, and one of the limitations for treating the renal tubular deposits seems to relate to a restricted glomerular filtration of the recombinant α-Gal A, which is a dimeric glycoprotein with a molecular weight of 110 kD. The recombinant enzyme contains several Mannose-6-phosphate (Man-6-P) residues (6). However, the molecular mechanisms for cellular uptake of recombinant α-Gal A are unknown, and the proximal tubule seems to express only small amounts of the cation-independent Man-6-P receptor at the apical membrane (7). In the past decade, however, it has become increasingly clear that the two endocytic receptors megalin (8) and cubilin (9), which are localized in the endocytic apparatus of the proximal tubule, are responsible for the reabsorption of the vast majority of proteins that are filtered in the glomeruli (10).

The aim of this study was to determine the normal distribution of α-Gal A in human kidney and to investigate the renal distribution of recombinant α-Gal A in mouse kidney after intravenous administration of the enzyme at therapeutic dosages. The renal pattern of proteinuria was studied in detail, and receptor-mediated cellular uptake of recombinant enzyme was analyzed.

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Materials and Methods

Preparation of Renal Tissue

Normal, uninvolved human renal tissue that was obtained from resected renal carcinoma kidneys and a from renal biopsy from a 62-yr-old female patient who had Fabry-Anderson disease and was not in treatment at the time for the biopsy was fixed in 4% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). The tissue was trimmed into small blocks, further fixed by immersion for 1 h in 1% formaldehyde, infiltrated with 2.3 M sucrose that contained 2% formaldehyde for 30 min, and frozen in liquid nitrogen. At the time for the biopsy, just before start of ERT, this patient had a plasma creatinine of 86 μM/L, GFR of 84 ml/min per 1.73 m² (51Cr-EDTA), and a urinary protein excretion of 2.0 g/24 h.

Human kidney sections were also obtained from paraffin-embedded blocks. α-Gal A–deficient mouse kidneys and normal mouse kidneys were fixed by perfusion retrograde through the aorta with 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). The tissue either was trimmed into small blocks, further fixed by immersion in 1% paraformaldehyde, infiltrated with 2.3 M sucrose for 30 min, and frozen in liquid nitrogen or was prepared for paraffin embedding. For morphologic and autoradiography studies, tissues were fixed in 1% glutaraldehyde, postfixed in 1% OsO4, dehydrated, and embedded in Epon. All animal experiments were approved and carried out in accordance with provisions for the animal care license provided by the Danish National Animal Experiments Inspectorate and the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No. 86-23). Ethical approval for the human studies was granted by the Copenhagen Local Research Ethics Committee, and informed consent was obtained from each patient.

Antibodies and Proteins

Affinity-purified rabbit polyclonal anti-(α-α-Gal A was provided by Genzyme Corp. (Framingham, MA). Sheep a-rat megalin (11) and rabbit a-rat cubilin (12) were provided by Dr. Pierre Verroust (INSERM U538, Paris, France). Rabbit a-human vitamin D–binding protein (DBP), rabbit a-human cathepsin D, rabbit a-human retinol-binding protein (RBP), rabbit a-human albumin, and rabbit a-human transferrin were from DAKO A/S (Glostrup, Denmark). For double-labeling experiments to identify lysosomal structures, we used a polyclonal anti-rat cathepsin B (Upstate Biotechnology, Lake Placid, NY) and a-cathepsin D from DAKO. Peroxidase-conjugated secondary antibodies were purchased from DAKO. Fluorescence-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, OR). Controls for unspecific binding were performed with nonspecific rabbit, goat, or sheep IgG from DAKO. Gold-conjugated goat anti-mouse and goat anti-rabbit IgG secondary antibodies were from British BioCell Int. (Cardiff, UK). Recombinant receptor-associated protein (RAP) was provided by Morten Nielsen (Department of Medical Biochemistry, University of Aarhus). Recombinant α-Gal A (Fabrazyme) was provided by Genzyme Corp.

Immunocytochemistry

For light microscopy, 0.8-μm cryosections were obtained at –80°C, and for electron microscopy, 70- to 90-nm sections were obtained at –100°C with an FCS Reichert Ultracut 5 cryoultramicrotome as described previously (13) and 2-μm paraffin sections were obtained with a Leica RM2165 microtome (Leica, Wetzlar, Germany). For immunolabeling, the sections were incubated with primary antibodies as indicated at room temperature for 1 h after preincubation in PBS that contained 0.05 M glycine and 1% BSA. The sections were subsequently incubated with peroxidase-conjugated secondary antibody (Dako), the peroxidase was visualized with diaminobenzidine, and the sections were subsequently counterstained with Maier’s stain for 2 min, or, for immunofluorescence, the sections were incubated with fluorescence-conjugated secondary antibodies as indicated. The sections were examined in a Leica DMR microscope that was equipped with a Leica DFC320 camera. Images were transferred by a Leica TFC Twain 6.10 program and processed using Adobe Photoshop 8.0. For EM immunolabeling the section were incubated with primary antibodies at 4 °C over night followed by incubation at room temperature for 1 h with 10 nm gold particles coupled to relevant immunoglobulins (BioCell, Cardiff, UK). The cryosections were embedded in methylcellulose containing 0.3% uranyl acetate and studied in a Philips CM100 electron microscope (Eindhoven, The Netherlands). Controls. Sections were incubated with secondary antibodies alone or with nonspecific IgG.

Immunofluorescence Microscopy of BN16 Cells

Rat yolk sac carcinoma (BN16) cells (14) were cultured as described previously (15,16). Briefly, BN16 cells were grown in 25-cm² plastic culture flasks (Corning Costar, Badhoevedorp, Holland) in Eagle’s minimal essential medium (Bio-Whittaker, Walkersville, MD) supplemented with 10% FCS (Biologic Industries, Fredensborg, Denmark), 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin (Bio-Whittaker) in a humidified atmosphere of 5% CO₂-95% air at 37°C. The cells were subcultured every fourth day with a split ratio of 1:5 by using 0.02% EDTA and 0.05% trypsin (Bio-Whittaker). Experiments were carried out with confluent monolayers of BN16 cells cultured in 24-well plates (Nagle Nunc International, Herford, UK) for uptake studies. Immunofluorescence on BN16 cells was performed essentially as described previously (17). In brief, after incubation with α-Gal A at indicated times, the cells were fixed with 1% paraformaldehyde for 1 min, permeabilized with Triton X100, and preincubated with PBS (0.01 M [pH 7.4]) that contained 1% BSA for 15 min. The cells were then incubated with rabbit α-Gal A. Subsequently, the cells were incubated with FITC-coupled swine anti-rabbit (Dako) or Alexa fluor 488 (Molecular probes). Rabbit and sheep sera were used as controls.

Autoradiography

For autoradiographic studies, mice were administered an intravenous injection of 120 μl of 125I-labeled α-Gal A (8.8 μg, approximately 18 × 10⁶ cpm, free iodine, 3.2%, iodinated by the chloramine-T method as described previously [18]). After 2, 4, or 24 h, the kidneys were fixed as described previously, and the tissue was postfixed, dehydrated, and embedded in Epon. The tissue was prepared for light microscope autoradiography on 1-μm-thick Epon sections using Ilford K2 emulsion (Essex, UK). The sections were exposed for 2 to 4 wk and developed in Kodak D19 developer (Balderup, Denmark). For electron microscope autoradiography, Ilford L4 emulsion was applied to 60-nm sections as described previously (19), and sections were exposed for 2 to 4 mo before development in Kodak D19 developer. The sections were studied in a Philips CM 100 electron microscope.

Tissues and Urine Samples

α-Gal A–deficient mice produced by gene targeting as described previously (5), and BALB/cA Bom mice were kept on a normal diet with free access to drinking water before infusion of recombinant α-Gal A (Fabrazyme) 1 mg/kg body wt. Urine was collected from the bladder immediately before perfusion of the mice and immediately frozen. Urine specimens were collected from patients with Fabry-Anderson disease (four male patients aged 15 to 37 yr and eight female patients aged 15 to 63 yr). In addition, urine samples were obtained from patients with Fabry-Anderson disease immediately before and 2 h after...
enzyme replacement infusions, 1 mg/kg body wt recombinant α-Gal A (Fabrazyme), and frozen until processed for immunoblotting. Mouse kidney cortical homogenates were obtained from the mice after perfusion of the kidneys through the abdominal aorta with saline. The kidney cortex was dissected from individual kidneys, minced, and homogenized in an ice-cold buffer (0.3 M sucrose, 25 mM imidazole, 8.5 μM leupeptin, 1 mM EDTA, 1 mM PMSF [pH 7.2]) for 30 s with an IKA Ultra-Turrax T8 homogenizer (IKA Labortechnik, Staufen, Germany). The homogenate was centrifuged at 4000 × g for 15 min and rehomogenized, and the supernatants were collected. All procedures were carried out on ice or at 4°C. BN16 cell homogenates were obtained similarly after incubation with α-Gal A at the indicated times, and the cells were scraped and processed.

**Surface Plasmon Resonance Analysis**

For the surface plasmon resonance (SPR) analyses, the BIAcore sensor chips (type CM5; Biosensor, Uppsala, Sweden) were activated with a 1:1 mixture of 0.2 M N-ethyl-N’-(3-dimethylaminopropyl) carbodiimide and 0.05 M N-hydroxysuccinimide in water according to the manufacturer. Rat cubulin and megalin purified by intrinsic factor-B12 and RAP affinity chromatography, respectively, were immobilized as described previously (20). The SPR signal from immobilized cubulin generated BIAcore response units (RU) that were equivalent to 25 to 35 fmol/mm². The flow cells were regenerated with 20 μl of 1.5 M glycine-HCl (pH 3.0). The flow buffer was 10 mM Hepes, 150 mM NaCl, 1.5 mM CaCl₂, and 1 mM EGTA (pH 7.4). A solution containing 1% BSA was also used to block the surface for 10 min. The specific binding of the probes was calculated by dividing the ratio RU ligand/mass receptor.

α-Gal A ELISA assay was performed using a rabbit polyclonal antibody to human α-Gal A. A 96-well plate was coated with 0.2 μg of rabbit polyclonal antibody to human α-Gal A. Urine samples were diluted 1:10, 1:50, 1:500, and 1:1000 and incubated in the wells that contained the antibody. Bound α-Gal A was detected using biotinylated anti-human α-Gal A antibody and streptavidin–horseradish peroxidase with O-phenylenediamine as substrate (Sigma, St. Louis, MO). α-Gal A activity assay was performed using 4-methylumbelliferyl-α-D-galactoside (Sigma) as substrate. Urine samples were analyzed for activity with undiluted and at 1:10, 1:100, and 1:1000 dilutions. The samples were then incubated in 27 mM citric acid and 46 mM sodium phosphate dibasic buffer (pH 4.4). For inhibition of endogenous urine activity of α-Gal B or N-acetylgalactosaminidase, N-acetylgalactosamine was included in the assay at a concentration of 117 mM. The amount of α-Gal A was calculated against recombinant human α-Gal A standards, and the results are shown as average of different dilutions.

**ELISA and Enzyme Activity**

**SDS-PAGE and Immunoblotting**

Samples of renal cortical homogenates, BN16 cell homogenates, or urine were mixed with Laemmli sample buffer that contained 2.5% SDS, subjected to SDS-PAGE using polyacrylamide minigels (Biorad Mini Protein II, Hercules, CA), and transferred to nitrocellulose paper. All urine blots were loaded according to urine creatinine. Blots were incubated for 1 h with horseradish peroxidase–conjugated secondary antibody diluted 1:3000. After a final wash, antibody binding was visualized using ECL enhanced chemiluminescence system (Amersham International, Bucks, UK). Controls involving incubation without primary antibody and incubation with nonspecific serum revealed no significant labeling.

**Results**

**Distribution of α-Gal A in Normal Human Kidney**

The lysosomes of proximal convoluted tubules were intensively stained for α-Gal A (Figure 1, A and B). Labeling was also very pronounced in thick ascending limbs (TAL; Figure 1, B and C) and in interstitial cells (Figure 1B). Glomerular cells including podocytes and parietal epithelial cells of the Bowman’s capsule were unlabeled, and macula densa cells exhibited very little labeling (Figure 1B). In medullary rays TAL and pars recta of proximal tubules were intensively labeled (Figure 1C). In the medulla (Figure 1D), collecting ducts and thin limbs of Henle were strongly labeled. Throughout the kidney, no staining of vascular endothelial cells was observed, as opposed to the strong staining that was seen in endothelial cells of liver sinusoids (data not shown). That the tubular staining for α-Gal A was indeed lysosomal was confirmed by double immunofluorescence staining for cathepsin B (Figure 1E).

**Figure 1.** Horseradish peroxidase immunohistochemistry for α-galactosidase A (α-Gal A) in normal human kidney. (A) Proximal tubule labeling. (B) Labeling in proximal tubules (P) and distal tubules (D). Macula densa cells (MD) and glomerulus (G) show no labeling. Arrows indicate labeled interstitial cells. (C) Strongly labeled pars recta of proximal tubule (P) and thick ascending limbs of Henle (TAL) in medullary ray are seen. (D) Labeling of collecting ducts (CD) and thin limbs (TL) in renal inner medulla. (E) Immunofluorescence for α-Gal A (green) and cathepsin B (red) in renal proximal tubules. Yellow stain indicates co-localization. Bars as indicated.
Urinary Protein Excretion in Patients with Fabry-Anderson Disease

Figure 2 demonstrates the urinary excretion of four proteins in 12 patients with Fabry-Anderson disease. One male and one female patient have very significant excretion of albumin, transferrin, vitamin D–binding protein [DBP], and retinol-binding protein [RBP]. In addition, two female patients and two male patients have intermediate excretions of the same proteins. It is notable that the excretion pattern is the same for all proteins investigated, including the 20-kD low molecular weight protein RBP and 50-kD DBP. For comparison, Western blots (data not shown) from a patient with minimal-change disease (urinary protein excretion 7 g/24 h) showed a very similar pattern of protein excretion.

The urinary excretion of α-Gal A was investigated in patients in ERT before infusion of the enzyme and 2 h after. As illustrated (Figure 3) for the two male and two of the female patients, there was a highly significant increase in urinary excretion of the infused enzyme, indicating glomerular filtration of the enzyme in these patients.

Biopsy of Female Patient with Fabry-Anderson Disease

A renal biopsy from the female patient with the intense proteinuria demonstrated in Figure 2, taken before ERT was instituted, revealed intensive accumulation of Gl3 in podocytes, parietal epithelial cells of Bowman’s capsule, distal tubule cells, vascular endothelial cells, and arteriolar wall cells (Figure 4, A through F). Although the accumulation was not observed in proximal tubule at the light microscopic level (Figure 4C), electron microscopy revealed a significant increase in lysosomal myelin figures (Figure 4F) but not as significant as in podocytes (Figure 4E). An analysis of the expression in this patient of the two endocytic receptors megalin and cubilin, the lysosomal enzyme cathepsin D, and reabsorbed vitamin D carrier protein DBP revealed no obvious changes (Figure 5).

Uptake of Recombinant α-Gal A in Rat Yolk Sac Cells

Immortalized rat yolk sac cells that expressed high amounts of megalin and cubilin endocytosed avidly the α-Gal A (Figure 6A). The accumulated enzyme was found after 2 and 5 h but was not detectable after 24 h (Figure 6B). The uptake was highly reduced after addition of RAP, a ligand for megalin (19), inhibiting binding of virtually all ligands for megalin (10) (Figure 6C). RAP-inhibitive binding to megalin of α-Gal A was demonstrated by SPR analysis with a Kd of approximately 600 nM (Figure 6C). Cubilin did not bind α-Gal A (results not shown).

Renal Uptake of 125I-Labeled α-Gal A in Mouse

After intravenous infusion of labeled α-Gal A, 1.1, 0.9, and 0.3% of the injected enzyme was found in the kidney after 2, 4, and 24 h, respectively, and 20, 20, and 12%, respectively, was found in the liver at the same time points. Autoradiography (Figure 7) revealed significant accumulation of the enzyme in
lysosomes of proximal tubules by light and electron microscopic autoradiography.

**Urinary Excretion and Renal Accumulation of Intravenously Infused Recombinant α-Gal A into Mice with Fabry-Anderson Disease**

After infusion of recombinant enzyme into α-Gal A gene-deleted mice, the urinary bladder was emptied just before perfusion fixation of the kidney. Analyses of the urine by Western blotting and ELISA and by measurements of the enzyme activity revealed that significant amounts of enzymatically active enzyme were excreted in the urine. Relatively high enzyme activity per unit of α-Gal A protein was observed in earlier time points compared with that from later time points, indicating the loss of activity when α-Gal A was present longer in urine (Figure 8).

The enzyme that accumulated in lysosomes of renal proximal tubules was visualized by immunoperoxidase, immunofluorescence, and immunogold techniques (Figure 9, A through C). Immunolabeling was found after 2, 4, and 24 h (Figure 9, A through H). In addition, labeling was seen in interstitial cells and in glomerular podocytes (Figure 9, D and G) but not in renal vascular endothelial cells as opposed to an intense uptake in hepatic sinusoidal endothelial cells in which accumulation was observed up to 24 h after infusion of α-Gal A (data not shown). No labeling was seen in noninjected mice with Fabry-Anderson disease or in absorption controls of injected mice.

**Discussion**

This study demonstrated the distribution of α-Gal A in the normal human kidney. The enzyme was found in lysosomes of cells from all parts of the nephron, collecting ducts, and interstitial cells. However, surprising, we were unable to demonstrate the enzyme in glomerular podocytes, parietal epithelial cells of Bowman’s capsule, or renal vascular endothelial cells—cells that are the main involved targets for accumulation of Gl3 in Fabry-Anderson disease. Whether these cells do not express the enzyme at all or are just below the detection limit for our methods remains to be answered.

The proteinuria that was seen in the 12 patients with Fabry-Anderson disease studied revealed an interesting pattern of a combined glomerular and tubular proteinuria as shown by the increased excretion of albumin, transferrin, DBP, and RBP. These proteins all are ligands for the proximal tubule endocytic receptors megalin and cubilin (for a recent review, see reference [10]), and the excretion pattern most likely indicates that when increasing amounts of higher molecular weight proteins such as albumin and transferrin start to appear in the tubular fluid, they start to compete for receptor binding of lower molecular
weight proteins such as DBP and RBP, which normally are filtered in relatively high amounts. This is also supported by our finding of a very similar pattern of protein excretion in a patient with minimal-change disease. The immunocytochemical observation of a seemingly unchanged distribution of megalin and cubilin, the lysosomal enzyme cathepsin D and DBP in the renal biopsy (Figure 4), does not contradict these findings because the immunocytochemical method is a relatively insensitive method for the detection of quantitative differences.

The glomerular filtration barrier for proteins has generally been considered to be in the range of albumin, which has a glomerular filtration fraction of \(0.01\) (21). However, we have shown that transferrin, 80 kD, is filtered to some extent normally both in human kidney and in rats and mice (22), and recently a 110-kD pancreatic lipase was found in the urine of healthy humans (23). These results support the observations of this study of a limited but significant glomerular filtration of recombinant \(\alpha\)-Gal A in \(\alpha\)-Gal A–deficient mice and patients, and this filtration is probably even more pronounced in patients with Fabry-Anderson disease and proteinuria. The seeming discrepancy of urinary excretion of enzyme shown in Figure 8, B (ELISA) and C (enzyme activity), in noninjected mice is most probably because we could not totally inhibit endogenous enzyme activity of urinary \(\alpha\)-Gal B.

Our results strongly indicate that megalin is the receptor that mainly is responsible for the proximal tubular accumulation of \(\alpha\)-Gal A. Although the proximal tubule cells are not generally considered to be involved in a pronounced way in Fabry-Anderson disease, probably at least in part because of their relatively fast turnover (24), the ERT probably has a beneficial effect on these cells, because as shown in this study (Figure 4F), myelin figures also to some extent can be found in proximal tubule cells of patients with Fabry-Anderson disease. The beneficial effect of ERT that was shown for more distal segments of the nephron and collecting duct can also be explained from the results of this article. Although the proximal tubule accumulated large amounts of filtered recombinant enzyme as shown in our mouse experiments, significant amounts were still excreted in the urine both in treated patients and in mice. Therefore, the tubular segments that are located farther downstream would also be exposed to the enzyme. Although we could not demonstrate administered enzyme in these cells, it is widely known that endocytic uptake of protein takes place in distal tubule cells as well as in collecting ducts probably by fluid-phase endocytosis (25–27). This uptake, although small, would also explain why these cells are slowly cleared (months) for Gl3 after ERT (4). In a study by Stern et al. (28), it was shown using immortalized renal epithelial cells that basolateral uptake far exceeded apical uptake in three different cell lines, including cells of proximal tubule origin. Our in vivo results do not support a significant basolateral uptake, at least in proximal tubules. However, these authors (28) also found that only in distal tubule cells did Man-6-P block uptake to any degree.

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**Figure 5.** Expression of megalin (A), cubilin (B), cathepsin D (C), and reabsorbed DBP (D) as shown by immunocytochemistry on cryosections from biopsy from the same patient as in Figure 4. No obvious changes from normal are observed. Bar as indicated.

**Figure 6.** (A) Immunofluorescent demonstration of uptake of \(\alpha\)-Gal A in BN16 cells after exposure to \(\alpha\)-Gal A (15 \(\mu\)g/ml for 1 h). (B) Western blots for \(\alpha\)-Gal A in BN16 cells after exposure for 1 h and chase incubation for 2, 5, and 24 h, left and right, inhibition by 1 \(\mu\)M receptor-associated protein (RAP). (C) Surface plasmon resonance analysis of \(\alpha\)-Gal A binding to megalin and inhibition of binding by RAP. The binding curve at the right was corrected for the binding of RAP itself to megalin.
suggesting that other receptors might be responsible for uptake in agreement with our results.

The small uptake of recombinant α-Gal A that was observed in podocytes in this study seems important for the slow clearance of Gl3 that was observed in these cells (4). However, the uptake mechanism for α-Gal A in these cells remains to be established because megalin has been demonstrated only in podocytes of Lewis rats (29) but not in any other species, including human. It should also be emphasized that even larger molecules, such as ferritin (440 kD), have been shown to be filtered in rat renal glomeruli and taken up by endocytosis in podocytes (30).

As shown in this study, renal interstitial cells intensively accumulated intravenously administered α-Gal A, which might explain the relatively rapid Gl3 clearance from these cells that was observed in ERT patients with Fabry-Anderson disease (4). The uptake mechanism for these cells is different from the proximal tubular uptake because these cells do not express megalin but could very well be via the Man-6-P receptor. The renal endothelial clearance that is observed in patients, however, is difficult to explain from these results. Although we saw high uptake of intravenously administered α-Gal A in hepatic endothelial cells (results not shown), we did not find uptake in renal endothelial cells. A possible explanation for the clearance is a rapid turnover of renal endothelial cells, which has also been demonstrated at least for the capillary endothelial cells (24) possibly combined with the lowered circulating amounts of Gl3 as a consequence of the ERT.

Conclusion
This study demonstrates the normal renal distribution of α-Gal A in lysosomes of virtually all tubular cells and interstitial cells but, surprising, not in renal endothelial cells and podocytes. ERT results in a certain urinary excretion of recombinant enzyme, illustrating that glomerular filtration takes place, and experiments with mice with α-Gal A–deficient Fabry-Anderson disease demonstrate uptake of the enzyme in podocytes, proximal tubules, and interstitial cells. Furthermore, the pattern of proteinuria shows increased urinary excretion of carrier proteins in patients with distinct glomerular proteinuria, carrier proteins that are normally totally reabsorbed.

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Disclosures
None.
References


Figure 9. Immunocytochemistry of kidneys from α-Gal A knockout mice that were administered an intravenous injection of α-Gal A. (A through E and G) Data from mice that were administered an injection 2 h before fixation. (F) Data from mice that were administered an injection 4 h before fixation. (H) Data from mice that were administered an injection 24 h before fixation. (A) Light microscopy of cryosection demonstrating avid uptake of enzyme in proximal tubules. (B) Electron microscopic immunogold labeling on cryosection showing proximal tubular lysosomal accumulation. (C) Double-labeling immunofluorescence (paraffin section) for α-Gal A (green) and cathepsin D (red). Yellow color indicates co-localization in lysosomes of the two enzymes. (D) Paraffin section demonstrating accumulation of α-Gal A in interstitial cells in outer stripe of outer medulla. (E) Initial part of proximal tubule (P) is labeled, and it seems that podocytes (arrows) are labeled. (F) At 4 h, labeling is seen at the initial part of proximal tubule. (G) At the electron microscopic level, lysosomal accumulation is seen in podocyte after 2 h (arrow). BM, glomerular basement membrane. (H) Even after 24 h, immunoreactive enzyme could still be found in proximal tubules. Bars as indicated.


