Abstract The inherited deficiency of arylsulfatase A (ASA) causes lysosomal accumulation of sulfoglycolipids (mainly sulfo-galactosylceramide, S-GalCer) and leads to metachromatic leukodystrophy in humans. Among visceral organs, kidneys are particularly affected. In the present study, the regional distribution and temporal development of sulfoglycolipid storage in kidneys of ASA-/- mice was investigated histochemically (alcian blue) and ultrastructurally. Furthermore, the sulfoglycolipid storage was examined in kidneys of double-knockout mice, which are incapable of: (a) degrading any sulfolipids (ASA-/-) and (b) synthesizing the major sulfolipid S-GalCer because of deficiency for galactosylceramide synthase (CGT), with the aim to search for additional ASA substrates. In ASA-/- mice, the nephron segments could be ranged in the order of decreasing sulfolipid storage: thin limbs of long-looped nephrons ~ thick ascending limbs > distal convoluted tubules > collecting ducts ~ short thin limbs. Macula densa and proximal tubules were unaffected. In ASA-/-/CGT-/- mice, the long thin limbs and distal convoluted tubules resembled those of ASA-/-/CGT+/- mice, while the other segments showed less storage. The results suggest that the turnover of sulfolipids in general is highest in the distal nephron except macula densa, and that long thin limbs and distal convoluted tubules are the main sites for turnover of a minor sulfolipid species, which is known to be synthesized in the kidney of CGT-/- mice.

Keywords Arylsulfatase A deficiency · Lysosomal sulfatide storage · Metachromatic leukodystrophy · Mouse kidney · Renal sulfolipids

Abbreviations For enzyme and lipids following Ishizuka (1997) and Tadano-Aritomi et al. (2000): ASA arylsulfatase A (E.C. 3.1.6.8) · CGT galactosylceramide synthase (UDP-galactose:ceramide galactosyltransferase; E.C. 2.4.2.62) · GalCer galactosylceramide · SB1a gangliotetraosylceramide II,IV3-bis-sulfate (HSO3-3Galβ-3GalNAcβ-4[HSO3-3]Galβ-4Glcβ-1Cer), identical with Stet2a of Trick et al. (1999) · SM3 lactosylsulfatide (HSO3-3Galβ-4Glcβ-1Cer) · SM4s sulfo-galactosylceramide (S-GalCer; HSO3-3Galβ-1Cer) · For nephron segments following Kriz and Bankir (1988): TL thin limbs · dTL descending thin limbs (of Henle’s loop) · aTL ascending thin limbs · TAL thick ascending limbs · DCT distal convoluted tubules · CD collecting ducts

Introduction

Metachromatic leukodystrophy (MLD) denotes a human lysosomal storage disorder which is due to the genetically determined deficiency of ASA. It is characterized by intralysosomal accumulation of sulfoglycolipids, mainly galactosyl sulfatide (sulfo-galactosylceramide, SM4s; for reviews see Kolodny and Fluharty 1995; von Figura et al. 2000). MLD is predominantly a neurological disease due to progressive demyelination. Yet, the sulfolipid storage is not restricted to neural tissues but occurs also in visceral organs among which the kidney is particularly affected (Wolfe and Pietra 1964; Gregoire et al. 1966; Martensson et al. 1966; Résibois 1971; Toga et al. 1972; Joosten et al. 1975; Burgess et al. 1985). This correlates with the high sulfoglycolipid content in normal kidney (for review see Ishizuka 1997). ASA-/- mice generated by genetic engineering resemble the late infantile type – the most severe type – of the human MLD as far as the absolute enzyme deficiency and failure to degrade sulfoglycolipids are concerned (Hess et al. 1996). One purpose of the present study was to investigate the regional distribution of normally stored sulfoglycolipids in the kidneys of ASA-/- mice. Sulfoglycolipids were visualized.
by use of cationic dyes. Additionally the ultrastructure and temporal development of the lysosomal storage were investigated.

The other purpose was to search for sulfoglycolipid storage in the kidneys of double-knockout mice which are incapable of: (a) degrading any sulfoglycolipids (ASA-/-), as explained above, and (b) synthesizing GalCer and its sulfated derivative SM4s. Such a mouse model with deficiency for CGT has previously been established (Coetzee et al. 1996). Since CGT-/- mice are yet able to synthesize minor sulfoglycolipids such as SM3 and SB1a (Tadano-Aritomi et al. 2000), it appeared of interest to examine whether and in which nephron segments ASA+/+/CGT-/- mice would develop sulfoglycolipid storage. The present results on ASA+/+ and ASA+/+/CGT+/+ mice allow some conclusions concerning regional differences of the sulfoglycolipid household in the individual nephron segments.

Materials and methods

ASA+/+ mice were generated by targeted disruption of the ASA gene as previously described (Hess et al. 1996). Twenty-seven adult mice (16 ASA+/+ mice and 11 wild-type mice) between 6 and 26 months of age and 19 young mice (14 ASA+/+ mice and 5 wild-type animals) on postnatal days (P) 2, 8, 15, 21, and 29 were investigated.

ASA-/- mice were kindly provided by T. Coetzee and B. Popko, Chapel Hill, N.C., USA. The animals were crossed with ASA+/+ mice. Genotyping was performed via Southern blotting as described (Hess et al. 1996; Coetzee et al. 1996). The kidneys of ASA+/+/-CGT+/+ mice (10 animals), ASA+/+/CGT-/- mice (11 animals), ASA-/-/CGT+/+ (6 animals), and wild-type mice (12 animals) were examined (ages 2 or 3 months). The animals were kept under conditions which were in accordance with the current German law on the Protection of Animals.

Tissue collection

Animals were deeply anesthetized with tribromoethanol (intraperitoneal injection), and killed by transcardial perfusion with 20 ml phosphate-buffered saline (PBS) and 60 ml fixative. For histochemistry, fixation was performed with Bouin’s solution diluted with PBS to 25% of the original formula; for electron microscopy 6% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) served as fixative. Some kidneys were taken before perfusion and shock-frozen in liquid nitrogen. For the sake of comparison, a formol-fixed kidney sample from a human case of the late infantile MLD was histochemically examined by the same technique as the murine kidneys. The sample was made available through the kind help of Dr. K. Harzer (Tübingen).

Detection of sulfoglycolipid storage

Sulfoglycolipids were detected by histochemical staining with the cationic dyes alcian blue (Alcian Blue; Aldridge, Steinheim, Germany) or cuprolinic blue (BDH Chemicals, Poole, UK) (Scott and Dorling 1965; Scott et al. 1981), as previously described (Scott et al. 2001). Briefly, after equilibration with the vehicle, small blocks were incubated with one of the dyes (0.05% dye in 0.025 M sodium acetate buffer, pH 5.7, containing 0.3 M MgCl2 and 1% paraformaldehyde) for 2–4 weeks. It may be noted that under appropriate binding conditions cuprolinic blue displays metachromasia, whereas alcian blue does not (Scott 1970; Juarranz et al. 1987).

Results

Observations on ASA-deficient mice

Control mice (wild-type)

In younger animals (<1 year), very few alcianophilic inclusions were seen in cells of the TL of Henle’s loop in the inner medulla; otherwise the neprhon was free of alcianophilic intracellular structures except for faint alcianophilic precipitates on the apical membrane of epithelial cells in the TAL. With increasing age the alcianophilic inclusions in the TL of the inner medulla became more frequent; on many occasions the affected tubular profiles could be identified as aTL (Fig. 1A). Ultrastructurally, the inclusions in TL epithelium were membranе-

Electron microscopy

Glutaraldehyde-fixed tissue samples were postfixed with 2% osmium tetroxide and embedded in Araldite or Epon. Epon embedding was essential for saving the large sulfolipid inclusions from tearing during ultrathin sectioning. Semithin sections were stained with toluidine blue, ultrathin sections were stained with uranyl acetate and lead citrate, and viewed with a Zeiss EM 900 electron microscope.

Fig. 1A–D Sulfoglycolipid storage in kidneys of adult mice as detected by means of alcian blue (pre-embedding incubation with the dye, paraffin sections, no counterstain). A ASA+/+ mouse (21 months of age), B–D ASA-/- mouse (11 months of age). Insets in A, C Kidney slices. Stereomicroscopic view, in buffer before postfixation with osmium tetroxide. In the ASA+/+ kidney, significant staining is seen only in the inner medulla (IM), which is known to contain high concentrations of sulfated proteoglycans in the extracellular matrix. OM Outer medulla. In the ASA-/- kidney, the inner and outer medulla and the medullary rays are intensely stained. A Transition between outer medulla (above) and inner medulla (below). Some cells in the ascending thin limb (aTL) of Henle’s loop contain alcianophilic inclusions, the thick ascending limb (TAL) is unstained. B Uppermost part of the inner stripe of outer medulla (cf. Fig. 2). The epithelium in the descending thin limbs of long-looped nephrons (dTL) and in TAL are packed with alcianophilic material, those in the descending thin limbs of short loops (dTL) and collecting ducts (CD) show moderate staining. C Transition outer/inner medulla. Extensive storage in aTL and TAL (not marked), moderate storage in CD. D Renal cortex. The TAL epithelium is filled with storage material, but the cells of the macula densa (MD) are unaffected. The glomerulus (G) and the proximal tubules (PT) are unstained. A–D ×620; insets ×64.