6 Abstract

An inherited deficiency for lysosomal enzyme Arysulfatase A (ASA) leads to 3-*O*-sulfo-galactosylceramide (sulfatide) accumulation and drastic demyelination in the central nervous system of humans. These cause the inherited lysosomal storage disease “metachromatic leukodystrophy” (MLD). As an animal model, the ASA-knockout mice have previously been generated by disruption of the ASA gene, such that ASA-knockout mice develop lysosomal sulfatid accumulation similar to the human MLD. Galactosylceramide (GalCer) and sulfatide are abundant sphingolipids in myelinating glial cells. Besides this, low levels of GalCer and sulfatide have been found in neurons. To date, it is not well understood, whether neuronal sulfatide is synthetized endogenously or rather, transported to neurons via an as yet unknown mechanism. In a transport model described by Han, sulfatide is transported by apolipoprotein E from astrocytes to neurons. In the present study it should be elucidated, whether the neuronal distribution of sulfatide storage in ASA- and ASA/ApoE-knockout mice differ in any way. We could show, that double-knockout mice as well as the ASA-knockout mice showed an increased sulfatide-level in the cortex and brain of 18-24 month old mice, which is to day that the additional ApoE-knockout did not cause any difference in neuronal sulfatide levels. By means of histochemical staining using alcian-blue it was proven that neuronal sulfatide storage was already detectable in relatively young mice of 12 months and intensified in older ASA- and ASA/ApoE-knockout mice. Neuronal sulfatide was most prominent in many nuclei of the medulla oblongata and pons in both, ASA- and ASA/ApoE-knockout mice, suggesting that the ApoE-deficiency does not have any different effect on the neuronal accumulation of sulfatide in ASA/ApoE-knockout mice as compared to ASA-knockout mice. It can therefore be concluded that sulfatide storage in neurons are either due to a completly different transport-protein or the accumulation is generated by endogenously synthesized sulfatide in neurons.

Transgenic mice overexpressing UDP-galactose:ceramide galactosyltransferase (CGT) under the control of the Thy1.2 promoter synthesize C18:0 fatty acid containing GalCer and sulfatide in neurons. Depending on the genetic background, these transgenic mice have a significantly reduced life span; e.g. mice on a C57Bl/6 background died earlier as compared to their counterparts on C57Bl/6/129Ola background. Transgenic mice were extremely sensitive to sound stimuli and displayed lethal audiogenic seizures after relatively mild acoustic stimulation, i.e. key

jangling (90-100 dB), which was reflected in a wild running phase followed by muscle spasms and partly occurring death. However, transgenic mice expressing cerebroside-sulfotransferase (CST) in neurons did not show sensitivity to audiogenic seizures. In contrast, transgenic mice expressing CGT and CST were characterized by an enhanced sensitivity to audiogenic seizure induction. The latter correlated with elevated sulfatide levels in neuronal plasmamembranes of double transgenic mice in comparison to CGT-transgenic mice, and strongly suggested that lethal audiogenic seizures are caused by elevated sulfatide levels in transgenic neurons. During the lysosomal storage disease MLD, neurons accumulate substantial amounts of sulfatide as well, which possibly contributes to the pathogenesis of the disease. CGT-transgenic mice can therefore be considered as a useful animal model to further investigate how sulfatide affects functional properties of neurons.

Even if there is no cure of the lysosomale storage disease MLD up until now, there are different approaches tested, of which substrate-reduction therapy currently seems to be the most promissing. Here, the synthesis of accumulating sulfatide is inhibited by specific small inhibitors, which are particularly modified to be able to cross the blood-brain barrier (BBB). In case of MLD, the very last step of sulfatide synthesis is the transfer of a sulphate group to galactosylcerebroside. This reaction is catalyzed by the CST, a type II transmembrane protein. Large amounts of purified CST are needed in order to allow detailed structural analysis and screening for specific inhibitors. However, the inefficient export of the enzyme from the endoplasmtic reticulum (ER) impedes high level purification of CST. Studies on different CST fusion-proteins indicated that high amounts of the CST are retained in the ER, which is most probably mediated by its luminal catalytic domain. In fact, a protein consisting of the amino-terminal membrane domain of CST fused to an EGFP reporter protein was efficiently transported to the Golgi Apparatus, whereas a fusion protein of the catalytic domain of CST with the amino-terminal membrane domain of dipeptidylpeptidase IV was retained in the ER. Interestingly, the expression of human sulfotransferases Gal3ST1, Gal3ST2 and Gal3ST4 demonstrated that their export from the ER was blocked, too. The relative amount of CST retained in the ER increased with the expression level of the protein, suggesting that an unknown ER export factor might limit ER export of the CST.