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The Role and Metabolism of Sulfatide in the Nervous System

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Abstract 3-*O*-sulfogalactosylceramide or sulfatide is a major component of the myelin sheath in the central and peripheral nervous system. The examination of mice deficient in the sulfatide-synthesizing enzyme, cerebroside sulfotransferase, provided new insight into the role of sulfatide in the differentiation of myelinating cells, formation of the paranodal junction, and myelin maintenance. Although in general regarded as a marker for oligodendrocytes and Schwann cells, sulfatide is also present in astrocytes and neurons. The relatively low amount of sulfatide in neurons can dramatically increase in the absence of the sulfatide-degrading enzyme, arylsulfatase A, as in metachromatic leukodystrophy. Recent advance in the understanding of this disease comes from studies on new transgenic mouse models. Significant changes in sulfatide levels have also been observed more recently in Alzheimer's disease and other diseases, suggesting that sulfatide might be involved in the pathogenesis of these diseases as well. This review summarizes recent studies on the physiological and pathophysiological role of sulfatide using transgenic mice deficient in its synthesis or degradation.

Keywords Alzheimer's disease · Galactosylceramide · Metachromatic leukodystrophy · Myelin · Oligodendrocytes · Schwann cells · Sphingolipids · Sulfatide

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Introduction

Glycosphingolipids are abundant components of cellular membranes in all eukaryotic cells. They play important roles in various physiological and pathophysiological processes (for a review, see [1–3]). A major fraction of glycolipids in the mammalian nervous system is build up by sulfoglycolipids. The first sulfoglycolipid in mammals was described by Thudichum in 1884 [4] who isolated the most abundant brain sulfoglycolipid, 3-*O*-sulfogalactosylceramide (sulfatide; SM4s; SGalCer), from human brain. Other sulfoglycolipids found in the mammalian brain are 3-*O*-sulfogalactosylglycerolipids (sulfogalactosyldiacylglycerol and the etherlipid/plasmalogen sulfogalactosylalkylacylglycerol or seminolipid, which is found at high concentration in the testis), and glycolipids carrying sulfated glucuronyl lactosaminyl residues [5, 6]. Mice deficient in this structure (HNK-1 epitope) have been generated, showing alterations in synaptic efficacy, learning, and memory [7, 8]. Because the HNK-1 structure is also a posttranslational modification of various adhesion molecules and other proteins, it is difficult to examine a specific role for lipid-linked HNK-1 structure using these mice. Other sulfolipids, like 3-*O*-sulfolactosylceramide (SLacCer) and 3-*O*-sulfoglucosylceramide (SGlcCer), are present in nonneuronal tissues or in the nervous system of some transgenic mice (as discussed below), but occur only at low concentrations or are absent in wild-type animals [6]. In the nervous system, sulfatide is enriched in the myelin sheath and makes up 4–6% of the myelin lipids [9, 10]. Together with its precursor galactosylceramide (GalCer), sulfatide accounts for almost one third of myelin lipids. As they are exclusively found on the extracellular leaflet of the membrane, up to two thirds of the outer surface of the myelin sheath is composed of sulfatide and

GalCer. As all sphingolipids, sulfatide exhibits variation of its structure because of different acyl chain lengths, which can also be hydroxylated (Fig. 1a). This structural variability shows a cell type-specific pattern, which at least in part can be explained by the differential expression of ceramide synthases [11–13] and fatty acid 2-hydroxylase [14–16].

The synthesis of sulfatide (Fig. 1b) starts at the lumenal leaflet of the endoplasmic reticulum by the transfer of galactose from UDP-galactose to 2-hydroxylated or non-hydroxylated ceramide, a reaction catalyzed by the UDP-galactose:ceramide galactosyltransferase [CGT (EC 2.4.1.45)] [17–19]. High concentration of UDP-galactose in the lumen of the endoplasmic reticulum is maintained by the specific interaction of CGT with the UDP-galactose transporter, retaining the latter in the endoplasmic reticulum [20]. After the transport of GalCer to the Golgi apparatus, sulfatide is finally synthesized by 3′-phosphoadenosine-5′-phosphosulfate:cerebroside sulfotransferase (CST; EC 2.8.2.11). CST has been cloned from human and mouse cDNAs [21, 22] and shown to be a homodimeric protein localized to a late Golgi compartment [23]. Degradation of sulfatide takes place in lysosomes where arylsulfatase A (ASA; EC 3.1.6.8) hydrolyzes the sulfate group. This reaction requires the help of a sphingolipid activator protein (saposin B [SapB]) that extracts sulfatide from membranes and thereby makes it accessible to ASA [24]. Accumulation of sulfatide because of the deficiency in ASA or (rarely) SapB causes the severe lysosomal storage disease metachromatic leukodystrophy (MLD). To date, it is widely accepted that the degradation of sulfatide and related 3-*O*-sulfoglycolipids essentially requires desulfation by ASA as the initial step, although the existence of other sulfatases has been proposed [25, 26]. An alternative, sulfatase-independent pathway of sulfatide degradation has been suggested recently by Zeng et al. [27] who found indications in a neuroblastoma cell line for the direct generation of ceramide from endocytosed sulfatide without prior desulfation.

ApoE-mediated Sulfatide Transport and Sulfatide in Nonmyelinating Cells

Although sulfatide is mainly found in oligodendrocytes and Schwann cells, low amounts have been detected in neurons and astrocytes [28, 29]. However, it is not clear whether sulfatide is synthesized by neurons or astrocytes themselves or imported, e.g., via lipoprotein endocytosis. Cultured purified astrocytes are capable of synthesizing (low amounts of) sulfatide, as shown by mass spectrometry [30, 31] and immunofluorescence using sulfatide-specific antibodies [28, 29, 31]. Isaac et al. [31] found significantly elevated relative levels of stearic acid (C18:0)-containing sulfatide compared to very long chain and 2-hydroxylated fatty acid containing sulfatides in the cortical gray matter of sulfatide-storing ASA-deficient mice (see below). Because neurons synthesize especially ceramides containing C18:0-fatty acids, because of high-level expression of stearoyl-CoA-specific ceramide synthase 1 (Lass1/CerS1) [13],

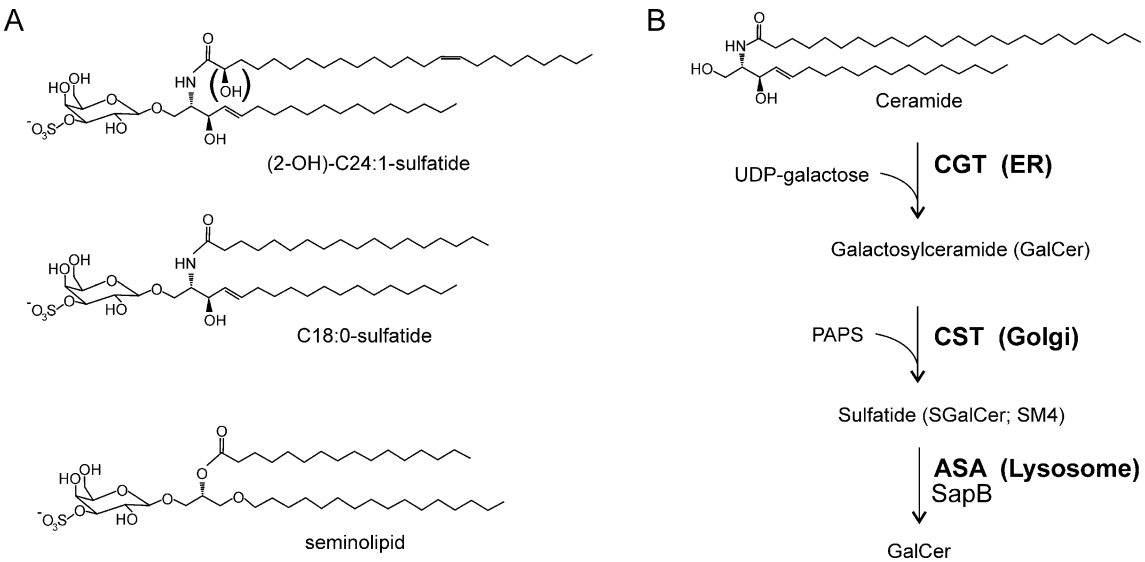

Fig. 1 **a** Structure of selected major and minor sulfogalactolipids in the mammalian nervous system. C24:1-sulfatide is the most abundant sulfatide in myelin. C18:0-sulfatide is found at higher concentration in cortical gray matter. The acyl chain of all sulfatide species can be 2-hydroxylated, as shown for C24:1-sulfatide. Seminolipid is a minor component of myelin. **b** Synthesis and degradation of sulfatide. *CGT* UDP-galactose:ceramide galactosyltransfearse (EC 2.4.1.45; gene name: *ugt8*), *CST* 3′-phosphoadenosine-5′-phosphosulfate:cerebroside sulfotransferase (EC 2.8.2.11; gene name: *gal3st1*), *ASA* arylsulfatase A (EC 3.1.6.8; gene name: *arsa*), *SapB* saposin B (gene name of the precursor prosaposin: *psap*)

presence of relatively large amounts of C18:0-sulfatide in gray matter would be in line with sulfatide synthesis by neurons. In contrast, myelin is enriched in very long chain fatty acid (C22/C24)-sulfatide. CGT expression is detectable in neuron subtypes of the spinal cord, brainstem, and cerebellum, but not in the forebrain [32]. Failure to detect CGT in some brain regions might be because of very low expression levels. The expression of CST appear to be less cell type-specific, and low levels of CST expression can be detected in various neurons [33]. Moreover, clear genetic evidence for CST activity in neurons comes from recent studies showing a selective increase in C18:0-sulfatide in most brain regions of transgenic ASA-deficient mice expressing CGT under a neuron-specific promoter [33].

Alternatively, sulfatide could be imported into neurons by receptor-mediated endocytosis of sulfatide-containing lipoproteins, as suggested by Han [34]. ApoE-containing lipoproteins play important roles in lipid homeostasis in the brain (for a review, see [35]). Sulfatide is present in ApoE-containing HDL-like lipoproteins in human cerebrospinal fluid (CSF), but not in ApoJ lipoproteins [30]. The main source of ApoE lipoproteins in brain are astrocytes [36]. Neurons express ApoE receptors, enabling them to endocytose ApoE lipoproteins [36, 37]. Endocytosis of ApoE lipoproteins is especially important to deliver cholesterol for axon growth and repair (for a review, see [38]). Sulfatide concentration in ApoE lipoproteins derived from murine astrocytes in culture, however, is below 1 nmol/mg, in contrast to around 50 nmol/mg in human CSF [30]. According to the model proposed by Han [34], ApoE-containing lipoproteins secreted by astrocytes take up sulfatide from myelin membranes by an unknown mechanism and are endocytosed by neurons via LDL receptor or LDL receptor-related proteins. However, because mice lacking both ASA and ApoE still accumulate significant amounts of sulfatide in neurons (M.E., unpublished observation), the ApoE-dependent pathway might not to be the only way to deliver sulfatide to neurons.

Role of Sulfatide in Differentiation of Myelinating Cells

Most sulfatide in the nervous system is present in oligodendrocytes and Schwann cells. During oligodendrocyte differentiation, sulfatide is first detected at the stage of immature oligodendrocytes and is upregulated before cells wrap myelin around axons, suggesting that sulfatide not only fulfills a role as a structural component of myelin [39, 40]. Previous studies using antibodies directed against sulfatide or CGT-deficient mice suggested that sulfatide acts as a negative regulator of oligodendrocyte differentiation [40–42]. This was confirmed by the analysis of CST-deficient mice [43]. Sulfatide deficiency leads to a twofold to threefold increase in the number of differentiated oligodendrocytes in brain and in vitro culture [43]. Thus, CST-deficient oligodendrocytes recapitulate the phenotype of CGT-deficient oligodendrocytes [42] and only sulfatide but not GalCer seems to act as an inhibitor of oligodendrocyte terminal differentiation. On the other hand, the initiation of myelination appear to be stimulated by sulfatide, at least in the case of cultured Schwann cells: sulfatide binds to components of the extracellular matrix, like tenascin-R [44] or laminin [45], which binds to signaling molecules at the glial membrane, e.g., F3 and integrins, and can generate signaling via c-src/fyn kinase [46]. $\alpha 6\beta 1$-integrins are the major laminin receptors of Schwann cells and form a signaling complex with focal adhesion kinase and fyn kinase required for the initiation of myelination [47]. Sulfatide synthesis by Schwann cells precedes the expression of laminins in the developing sciatic nerve, and high-affinity binding of sulfatide to laminin is sufficient to initiate basement membrane assembly and c-src/fyn kinase activation in cultured Schwann cells [46]. However, alternative, sulfatide-independent pathways must exist, as the initiation of myelination appear to proceed normally in CST-deficient mice. As binding of laminin-2 (present on axons in the CNS) to oligodendroglial $\alpha 6\beta 1$-integrins induces its coclustering with PDGFα receptor-containing lipid rafts to generate survival-promoting signaling [48], it is tempting to speculate that sulfatide might also play a role in regulating oligodendrocyte survival.

Role of Sulfatide at Axo-glial Junctions

The first animal studies to examine the role of sulfatide have been performed using CGT-deficient mice [49, 50]. Because these mice not only lack sulfatide, but are also deficient in GalCer and moreover show a number of secondary changes in the lipid composition of myelin, e.g., upregulation of 2-hydroxylated glucosylceramide and sphingomyelin [49–51], it was not possible to determine to what extent loss of sulfatide, GalCer, or the other changes are responsible for the phenotype of CGT-deficient mice. An important step toward understanding the role of sulfatide in the nervous system was, therefore, the generation of CST-deficient mice by Honke et al. [52]. CST-deficient mice lack sulfatide and sulfogalactoglycerolipids. It is important to note, however, that other lipids are not significantly altered in these mice [52].

CST-deficient mice produce normal compacted myelin [52], and the initiation of myelination appear to be normal [53]. However, whereas *g*-ratios of myelinated axons usually decrease with age whereas myelination proceeds, *g*-ratios in the CNS of CST-deficient mice remain almost

constant during development, leading to significantly thinner myelin sheaths in adult mice compared to wild-type controls [53]. Thus, myelin maintenance appear to be disturbed, as also indicated by redundant myelin, vacuolar degeneration, and uncompacted myelin in older CST-deficient mice [53]. These structural changes occur at significant lower frequency than in CGT-deficient mice, and in contrast to the latter, CST-deficient mice exhibit no signs of severe demyelination [52, 53], suggesting a sulfatide-independent role of GalCer. It cannot be ruled out, however, that increase in other lipids (for example, hydroxylated GlcCer and sphingomyelin) in CGT-deficient mice is responsible for the more severe phenotype. In addition to its function in myelin maintenance, sulfatide appears to affect glial–axon signaling as exemplified by reduced axon caliber in adult CST-deficient mice [53].

Axolemma sodium channel clusters at the nodes of Ranvier are formed normally in young CST-deficient mice [54], and the switch from $Na_v1.2$ to $Na_v1.6$ sodium channels in mature nodes occurs normally [55]. The maintenance of these clusters is, however, impaired as they decrease significantly with age [54]. Similarly, $K_v1.2$ channels move from the paranodal to the juxtaparanodal region [54]. This correlates with the disappearance of Caspr and neurofascin 155 (NF155) clusters, essential components of the axo-glial junction [54]. Mislocalization of ion channels might be because of the disruption of the paranodal axo-glial junctions. On the other hand, there is evidence that oligodendrocytes assist in sodium channel cluster maintenance independent of myelin [56]. Sulfatide is also essential for the maintenance of axo-glial contact in the peripheral nervous system. In CST-deficient peripheral nerves, extended axonal protrusions at the node of Ranvier contain abnormal enlarged vesicles and Caspr and NF155 clusters are unusually short or absent [57]. In addition to these structural alterations of the nodes and paranodes, the number of Schmidt–Lanterman incisures are increased, and elevated levels of Annexin II might indicate structural changes at the Schmidt–Lanterman incisures [58]. As a component of detergent-resistant myelin membranes (lipid rafts) [59], sulfatide could be involved in recruiting proteins to the myelin membrane. For example, analysis of CGT-deficient mice showed altered raft association of proteolipid protein [59]. Disrupted paranodal junctions might be explained by the impaired partitioning of adhesion molecules into lipid rafts. Formation of the paranodal junctions requires complex formation between axonal (Caspr and contactin) and glial adhesion molecules (NF155) [60]. Differentiation-dependent association of NF155 with lipid rafts correlates with the formation of paranodes during development and its raft association is reduced in CGT-deficient mice [61]. Thus, sulfatide (and/or GalCer) might be required to form and to recruit NF155 into stable lipid rafts. NF155 clusters will then concentrate Caspr and contactin in the axonal membrane, thereby forming stable axo-glial junction [60, 61].

To some extent, myelin and lymphocyte protein (MAL)-deficient mice exhibit similar alterations of the paranodal junctions in the CNS as observed in CST-deficient mice [62]. MAL, a tetraspan lipid raft-associated protein, is believed to play an important role in the formation of membrane microdomains in myelin [63]. In view of its binding to sulfatide [64] and the sulfatide-dependent missorting of MAL in sulfatide-storing kidney cells [65] (see below), MAL deficiency might impair the transport of sulfatide to "paranodal lipid rafts" [62] causing destabilization of the axon–glial junction.

Taken together, all available data show that sulfatide can modulate the differentiation of myelinating cells, but is not required for myelin formation per se and is not an essential structural component of compacted myelin (Table 1). Sulfatide is, however, involved in glial–axon interactions as demonstrated by its essential role in the maintenance of the paranodal axo-glial junction.

Pathology of Sulfatide Storage

MLD is a lysosomal storage disease caused by deficiency in ASA (for a review, see [66]). The incidence of the disease is between 1:40,000 and 1:70,000 at birth. In the absence of ASA activity, sulfatide accumulates in the nervous system, mainly in oligodendrocytes, Schwann cells, and phagocytes, but also in astrocytes and neurons. The inability to degrade sulfatide results in intralysosomal storage of the lipid. In the most common, early-onset, late infantile form of the disease, patients display gait disturbances and ataxia, later develop epileptic seizures, spastic quadriplegia, and eventually die in a decerebrated state. The hallmark of the late infantile form of the disease is a progressive loss of myelin. The late-onset, juvenile, and adult forms of MLD are milder because of residual ASA activity, and patients often exhibit psychiatric symptoms, often in the absence of significant demyelination.

ASA-deficient mice [67, 68] have been used for more than a decade as an animal model of MLD. Sulfatide storage pattern, as detected by alcian blue staining, in ASA-deficient mice closely resembles the sulfolipid storage pattern observed in MLD patients [69]. These mice develop clinical signs that resembles those of an early phase of human MLD, but they do not demyelinate [68]. Clear behavioral changes became detectable beyond 12 months of age in previous studies [67, 70, 71]. However, a recent study using more sensitive techniques to test neuromotor capabilities detected behavioral alterations already at 6 months of age [72], which correlates well with the

Table 1 Comparison of the effects of sulfatide deficiency and sulfatide accumulation in transgenic mice

Sulfatide deficiency	Sulfatide accumulation
Oligodendrocytes	
Enhanced terminal differentiation [42, 43]	Delay in myelin formation [73]
Node of Ranvier	
Impaired maintenance of sodium channel, potassium channel [54], caspr, and NF155 clusters [57], and disorganized myelin lateral loops at the node of Ranvier [52]	No structural abnormalities at the node of Ranvier reported
Myelin maintenance	
No demyelination [52]	Loss of myelin and hypertrophic peripheral neuropathy in aged mice with increased sulfatide synthesis [98]
Myelin gene expression	
Normal MAL[a] [117] and PLP [52] mRNA levels	Downregulation of MAL and PLP mRNA [65, 73]
Lipids	
No secondary changes in other lipids [52]	Reduced GalCer and cholesterol [73, 88] and increase in gangliosides GM3 and GD3 [76]
Neurons	
Neuronal degeneration [52] and reduced axon diameters [53], most likely secondary to myelin abnormalities	Degeneration of Purkinje cells [70]; axonal degeneration (in case of increased sulfatide synthesis in neurons) and cortical hyperexcitability [33], caused by neuronal sulfatide accumulation

[a] In CGT-deficient mice.

beginning of a significant sulfatide accumulation at this age, especially in the cerebellar white matter [72, 73]. Gait abnormalities are the earliest clinical signs in young ASA-deficient mice [72] as in human MLD subjects (for a review, see [66]).

ASA-deficient mice have been extensively used to evaluate therapeutical approaches for MLD, e.g., adeno-associated viral- and lentiviral-mediated gene therapy [74–76], gene therapy using hematopoietic stem cells [77–80], enzyme replacement therapy [81], and cell-based therapies using oligodendrocyte progenitors [82] or embryonic stem cell-derived oligodendrocytes [83]. These studies have been described in several recent reviews [84–87] and will, therefore, not be discussed in this paper.

How sulfatide accumulation leads to neurological symptoms, loss of myelin, or neuronal degeneration in some brain areas is largely unknown. Loss of neurons does not always correlate with sulfatide storage, as for example cerebellar Purkinje cells degenerate in old ASA-deficient mice without any indication for lipid accumulation [70]. Accumulation of sulfatide is not restricted to the lysosomal compartment, but was also observed in myelin [65]. This raises the important question to what extent intracellular lysosomal storage or elevated sulfatide levels in the plasma membrane are involved in the pathogenesis of the disease.

Secondary changes in lipids have been observed in ASA-deficient mice, as in other lysosomal storage disorders: increase in gangliosides G_{M2} and G_{D3} [76], an early decrease in GalCer [73], and reduced cholesterol levels in old ASA-deficient mice [88], i.e., changes which in part have also been described in human MLD [88–90]. Secondary changes in gangliosides occur in other lysosomal storage diseases and elevated G_{M2} levels correlate with ectopic dendritogenesis (for a review, see [91]). Although G_{M2} accumulation in ASA-deficient mice is only moderate, it might also affect neuronal function [76]. According to Saher et al. [92] high cholesterol levels are essential for myelin formation and maintenance. The slightly (15%) reduced cholesterol level in ASA-deficient mice [88] might thus well be relevant for the pathogenesis of the disease. As in human MLD, sulfatide storage is also detectable in neurons and astrocytes of ASA-deficient mice [69]. In addition to sulfatide accumulation, ASA-deficient mice show elevated levels of seminolipid, which, however, does not increase with age [93]. This might suggest a very low turnover of this lipid after the period of myelination or an unknown alternative pathway of seminolipid degradation.

MAL is a lipid raft-associated tetraspanin protein found in oligodendrocytes and Schwann cells [94] known to bind sulfatide [64]. Its mRNA and protein were found to be specifically downregulated in ASA-deficient mice [65]. The pathway leading to MAL mRNA downregulation is unknown. It is interesting to note that it is age-independent and thus does not correlate with the amount of accumulating sulfatide [73]. In contrast, MBP and PLP expression is transiently downregulated during the period of myelination, but reaches normal levels in older mice [73]. In the absence of a significant reduction in the number of mature oligodendrocytes, this might indicate the inhibition of signaling processes by sulfatide in fully differentiated oligodendrocytes [73]. MAL mRNA is also reduced in ASA-deficient Schwann cells upon treatment with sulfatide,

and this effect can be reversed by the additional endocytosis of ASA [95]. In kidney cells, sulfatide storage is accompanied by a mislocalization of MAL, which switched from the plasma membrane to a late endosomal/lysosomal compartment [65]. The redistribution of MAL and its colocalization with sulfatide can be explained by their specific interaction [64]. Whether MAL mistargeting also occurs in ASA-deficient Schwann cells or oligodendrocytes is currently not known.

Improved Animal Models of Metachromatic Leukodystrophy

A limitation of the conventional MLD mouse model is the lack of significant loss of myelin in both brain and peripheral nervous system [67, 68]. This might be because of species-specific differences in sphingolipid metabolism, as suggested for other storage disorders, e.g., Tay–Sachs disease [96]. A more likely explanation is, however, that the lower life span of mice prevents sulfatide accumulation in ASA-deficient mice to a level found in human MLD patients. This "disadvantage," however, also enables one to generate MLD mouse models with an increase in sulfatide storage selectively in different cell types, e.g., oligodendrocytes, Schwann cells, neurons, or astrocytes. It should be kept in mind that a cell type-specific knock-out of lysosomal enzymes is not feasible because they can be secreted and endocytosed by neighboring cells via mannose-6-phosphat receptor (for a review, see [97]).

An obvious way to improve the MLD mouse model was to increase sulfatide synthesis. This was done by transgenic overexpression of CGT and CST in ASA-deficient mice [33, 98]. It is interesting to note that the overexpression of CGT specifically in myelinating cells (using the proteolipid protein [PLP] promoter) of wild-type mice leads to unstable myelin and progressive loss of myelin, although changes in steady state levels of GalCer and sulfatide are only moderate [99]. In contrast, overexpression of CST under the PLP promoter does not affect myelin stability per se. In the absence of a functional ASA gene, however, CST overexpression causes a significant increase in sulfatide storage in the brain and peripheral nerves [98]. This is accompanied by loss of myelin in aged (18-month-old) CST-transgenic ASA-deficient mice in both peripheral and central nervous system. The pathology is more severe in the peripheral nervous system, which exhibits clear signs of a hypertrophic peripheral neuropathy, indicating repeated demyelination and remyelination events [98], and also observed in human MLD subjects [66]. Sulfatide storage in CST-transgenic ASA-deficient mice is increased in oligodendrocytes, Schwann cells, and especially in macrophages that had taken up myelin debris. The pathway of this uptake by macrophages is not understood. Storage material might be actively exocytosed, as shown for ASA-deficient kidney cells [100]. In summary, CST-transgenic ASA-deficient mice are the first animal model showing the typical hallmarks of (early-onset, infantile) human MLD.

The selective increase of sulfatide storage in neurons by transgenic overexpression of CGT under the control of the Thy1.2 promoter [33] also significantly affects clinical symptoms in ASA-deficient mice. Whereas these mice do not show signs of demyelination, they show a progressive axonal degeneration, which clearly correlates with the increase in neuronal sulfatide storage and motor coordination deficits. In addition, cortical EEG measurements reveal cortical hyperexcitability with recurrent spontaneous cortical discharges lasting 5 to 15 s in CGT-transgenic ASA-deficient mice and, albeit to a lesser extent, in "conventional" ASA-deficient mice [33]. It is interesting to note that cortical hyperexcitability does not correlate with the amount of storage material, but decrease with age. Decreased hyperexcitability might be the result of two effects: increased sulfatide storage and axonal degeneration. Alternatively, it is possible that elevated sulfatide concentrations in the neuronal plasma membrane (and not the intracellularly stored sulfatide) is responsible for the hyperexcitability and this concentration might not necessarily correlate with the total amount of accumulating sulfatide. Neurological symptoms in the absence of demyelination and peripheral neuropathy in these mice is reminiscent to late-onset, adult MLD. It is, therefore, possible that, in the late-onset forms of the disease, sulfatide accumulation in neurons contributes significantly to the pathogenesis. A brief summary of the main effects of sulfatide storage in mice is given in Table 1.

Overexpressing CST in ASA-deficient mice has only a minor effect on the sulfatide level, but leads to the appearance of SLacCer and other sulfoglycolipids [33]. Accumulation of these lipids, however, did not cause deterioration of the pathology [33]. Together with the fact that in the "conventional" MLD mouse model and in human MLD subjects [66], SLacCer is undetectable in the brain (according to Molander-Melin et al. [93], SLacCer in mouse brain is below 0.2 pmol/mg), this strongly suggests that SLacCer does not have a major impact on the pathogenesis of MLD. It also suggests that hyperexcitability and axonal degeneration might be "sulfatide-specific" and not (only) caused by jamming the endosomal–lysosomal system [101].

Hyperexcitability in mice accumulating sulfatide in neurons suggests modulation of electrophysiological properties by sulfatide. This might not only be relevant for the pathogenesis of MLD but it could also point to a physiological role of sulfatide in neurons. Sulfatide potentially could affect functional properties of membrane proteins, like ion channels, ion pumps, receptors,

or transporters. Sulfatide might affect receptor binding of neurotransmitters [66] and regulates fish gill Na^+/K^+-ATPase activity by its partitioning into sulfatide-enriched lipid rafts [102, 103], although it is not known if this is also true for mammalian Na^+/K^+-ATPase. Activation of voltage-gated potassium channels by sphingomyelinase showed that charged membrane lipids can affect functional properties of ion channels [104]. Recent work by several groups indicated the activation of potassium channels by sulfatide. Sulfatide increases opening probability of the large conductance calcium-sensitive potassium channel (BK_{Ca}) [105] and stimulates ATP-sensitive inwardly rectifying K^+ (K_{ATP}) channels in rat pancreas β-cells [106]. This K_{ATP} channel is a complex of Kir6.2 and SUR1 subunits (for a review, see [107]), which is also expressed in the brain and appear to play a role in the protection against ischemic neuronal injury and epilepsy [108]. These ion channels are the first candidates to test for their capacity to be modulated by sulfatide in the mammalian brain. In addition to its effect on ion channels, sulfatide could affect excitability by modulating dopamine uptake in synaptosomes [109].

Changes in Sulfatide Levels in Other Diseases

Elevated sulfatide levels in the CSF can indicate loss of myelin in MLD and other diseases. However, in some diseases, significant changes in sulfatide levels have been reported in the absence of significant myelin loss. Substantial decrease in sulfatide has been observed in a case of progressive epilepsy with mental retardation [110], which is caused by mutations in the *CLN8* gene, a member of the TLC (TRAM-Lag1-CLN8) domain-containing family with unknown function [111]. Members of this protein family include ceramide synthases and membrane proteins involved in protein translocation at the endoplasmic reticulum [112]. Elevated levels of sulfatide by 30–40% in the superior frontal and cerebellar gray matter were found in Parkinson's disease subjects [113]. The metabolic alterations causing changes in sulfatide levels in these diseases are currently unknown.

A substantial reduction in sulfatide levels has been observed in Alzheimer's disease in the brain and CSF [114, 115]. Cerebroside sulfotransferase activity is normal, suggesting that accelerated degradation causes sulfatide reduction [114]. Because of the potential role of ApoE lipoproteins in controlling sulfatide levels in the brain, sulfatide deficiency might be linked to ApoE lipoprotein metabolism [35]. The human ApoE ε4 allele is a genetic risk factor for Alzheimer's disease [116], and its expression in ApoE-deficient mice correlates with significantly reduced brain sulfatide levels [30]. Loss of sulfatide in Alzheimer's disease is most severe in the cerebral gray matter (>90% reduction) compared to about 50% in the white matter [114]. Because the majority of sulfatide is present in oligodendrocytes (in white and gray matter) and myelin, the massive loss of sulfatide can only reflect mainly the loss of sulfatide in oligodendrocytes and myelin, although reduced sulfatide levels in neurons or astrocytes cannot be excluded. Normal levels of other lipids, particularly GalCer and sphingomyelin [114], however, suggest that the loss of sulfatide is not because of a general defect in sphingolipid metabolism in oligodendrocytes or increased age-related myelin breakdown. It does not appear to be directly caused by β-amyloid accumulation [113] and is already apparent in very mildly impaired subjects [114]. The low levels of sulfatide in Alzheimer's disease is accompanied by a significant increase in ceramide [114], possibly because of increased sulfatide degradation, although other mechanisms have not been excluded. To what extent reduced sulfatide and increased ceramide levels contribute to the pathogenesis of the disease is an important question to address in the future.

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