Brain Pathology 8: 79-100(1998)

Recent Advances in the Biochemistry of Sphingolipidoses

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Glycosphingolipids are ubiquitous membrane components of eukaryotic cells. They participate in various cell recognition events and can regulate enzymes and receptors within the plasma membrane. Sphingolipidoses are due to an impaired lysosomal digestion of these substances. Glycosphingolipids are degraded by the action of exohydrolases, which are supported, in the case of glycosphingolipids with short oligosaccharide chains, by sphingolipid activator proteins. Five sphingolipid activator proteins are known so far, the GM2-activator and the SAPs, SAP-A to D (also called saposins). Degradation of glycosphingolipids requires endocytic membrane flow of plasma membrane derived glycosphingolipids into the lysosomes. Recent research focused on the topology of this process and on the mechanism and physiological function of sphingolipid activator proteins. Limited knowledge is available about enzymology and topology of glycosphingolipid biosynthesis. Recently, intermediates of this metabolic pathway have been identified as novel signalling molecules. Inhibition of glycosphingolipid biosynthesis has been shown to be beneficial in the animal model of Tay-Sachs disease. Mice with disrupted genes for lysosomal hydrolases and activator proteins are useful models for known human diseases and are valuable tools for the study of glycosphingolipid metabolism, the pathogenesis of sphingolipidoses and novel therapeutic approaches.

Sphingolipidoses

The sphingolipidoses are a group of inherited human diseases in which sphingolipids accumulate in one or more organs due to a degradation disorder. With the exception of Fabry's disease (19) they exhibit an autosomal recessive mode of inheritance. Symptoms and course of these diseases vary widely between forms with onset in early childhood and death within the first years of life on the one hand, and chronic forms on the other hand. The brain is affected in many of these disorders, although the function of the skin or visceral organs can be impaired in others. Their pathogenesis is poorly understood and a causal therapy is only available for the nonneuronopathic form of Gaucher's disease. The genes of most proteins involved in sphingolipid degradation have recently been cloned enabling genotype-phenotype correlation and facilitating the diagnosis of sphingolipidoses. In addition, animal models of many sphingolipidoses have been created by targeted disruption of the respective genes in mice. In the future, these animal models will serve as valuable tools for the investigation of the pathogenesis of these diseases and for the study of therapeutic approaches, including enzyme replacement, organ transplantation, gene therapy, and substrate deprivation.

Apart from the clinical aspects, there are many questions concerning the details of sphingolipid metabolism. Recent research focuses e.g. on the topology of lysosomal digestion, the physiological function of sphingolipid activator proteins, and the metabolism at membrane surfaces.

In contrast to their degradation, sphingolipid biosynthesis and function is much less understood. Sequence information has recently become available for some glycosyltransferases involved in the synthesis of complex glycosphingolipids (GSLs); but not for the enzymes catalyzing the early steps of this metabolic pathway. Inhibitors of biosynthesis have been described and might become useful in the treatment of sphingolipidoses as well as other pathologic conditions. Recently it became clear that lipid intermediates of sphingolipid metabolism serve as signalling molecules, e.g. as second messengers. Very little is known about the enzymology of these processes, their regulation and even the identity of the signaling compounds. It cannot be excluded that altered levels of these bioactive intermediates contribute to the pathogenesis of the sphingolipidoses.

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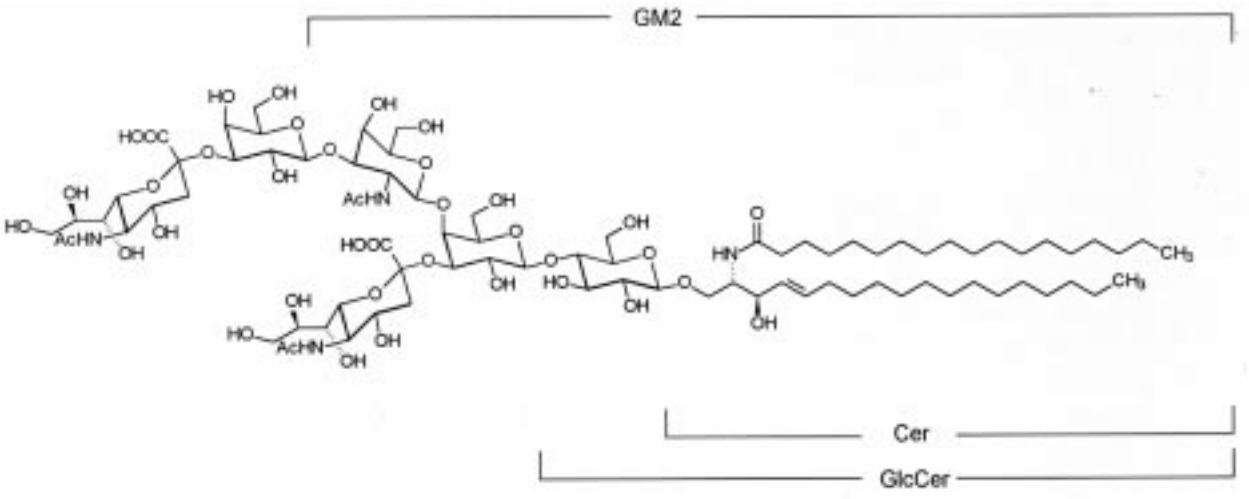

Figure 1. Structure of ganglioside GD1a, the most abundant sialic acid bearing glycosphingolipid in adult human brain. Abbreviations refer to partial structures: Cer - ceramide; GlcCer - glucosylceramide ; GM2 - ganglioside GM2.

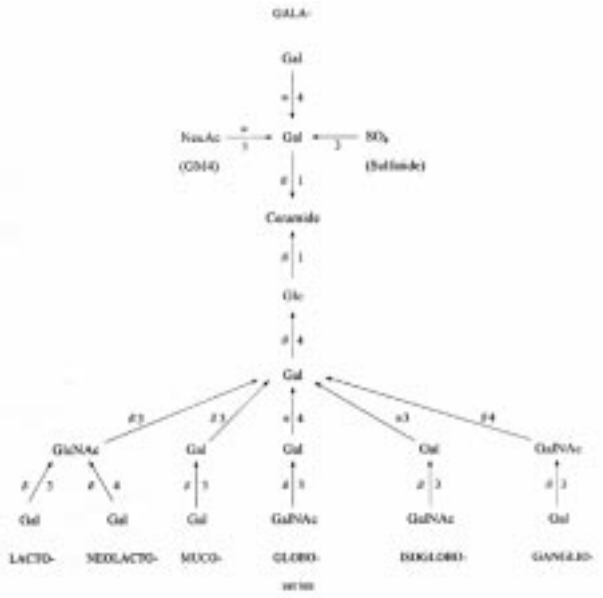

Figure 2. Structures and trivial names of selected glycosphingolipid series. Ceramide: N-Acylsphingosine; Gal: D-Galactose; GalNAc: N-Acetyl-D-galactosamine; Glc: D-Glucose; GlcNAc: N-Acetyl-D-glucosamine; NeuAc: N-Acetylneuraminic acid; SO4: Sulfate. The terminology used for gangliosides is that of Svennerholm (133).

Structure and function of glycosphingolipids

Glycosphingolipids (GSLs) are minor components of eukaryotic plasma membranes (80, 149). They contain a hydrophobic ceramide moiety that acts as membrane anchor and an extracellular orientated glycan chain (Figure 1). Ceramide itself consists of a long chain amino alcohol, D-*erythro*-sphingosine, which is acylated with a fatty acid. It is also a structural component of a plasma membrane phospholipid, sphingomyelin. GSLs are heterogeneous with respect to both, their carbohydrate and ceramide portion. Sphingolipids with unusual ceramide structures are found in the skin where they contribute to the epidermal water permeability barrier (44). Variations, especially in the type, number and linkage of sugar residues within the oligosaccharide chain give rise to the wide range of naturally occurring GSLs. More than 300 different structures have been characterized from natural sources (129). GSL structures depend on the species and can be classified into series which are characteristic for a group of evolutionary related organisms (Figure 2). Beside the species dependence, GSLs form cell-type specific patterns on the cell surface. In particular, sialic acid containing GSLs of the ganglio-series, the gangliosides, are abundant on neuronal cells (Figure 3). Several lines of evidence indicate that they contribute to brain function (66, 134), e. g. as modulators of neuronal calcium or of neuronal differentiation and development. Together with glycoproteins and glycosaminoglycans the GSLs contribute to the glycocalyx which covers the cell surface with a carbohydrate wall. GSLs form cell-type specific patterns at the surface of eukaryotic cells which change with cell growth, differentiation, viral transformation and oncogenesis (36). At the cell surface, they can interact with toxins (46), viruses (76), and bacteria (51). These pathogens take advantage of the close spatial neighborhood between specific carbohydrate recognition sites and the plasma membrane. Various physiolog-

ical events can be influenced by GSLs, e.g. embryogenesis, neuronal and leukocyte differentiation, cell adhesion, signal transduction and leukocyte differentiation. They interact with membrane bound receptors and enzymes (85) and are involved in cell type specific adhesion processes (95, 147). In addition, lipophilic products of GSL metabolism such as ceramide and sphingosine-1-phosphate play a role in signal transduction events (128). Finally, GSLs provide the exoplasmic leaflet of biological membranes with high chemical and mechanical stability (142) and protect them from inappropriate degradation and uncontrolled membrane fusion. Limited knowledge about the precise *in vivo* function of GSL is available today. A variety of observations indicate that they can participate in different biological events, but in most cases definite proofs for their importance are missing. In general, the conservation of the overall GSL structure during evolution and the absence of inherited diseases affecting GSL biosynthesis indicate their functional importance for the living organism.

Sphingolipid biosynthesis

GSLs and sphingomyelin reside predominantly in the exoplasmic leaflet of the plasma membrane. The enzymes involved in sphingolipid biosynthesis are membrane bound proteins. Little is known about their structure, catalytic mechanism, biosynthesis, and regulation. *De novo* biosynthesis of GSLs (141) takes place in the same intracellular compartments as glycoprotein biosynthesis and is coupled to intracellular vesicular transport of the growing molecules through the cisternae of the Golgi apparatus and to the plasma membrane. It starts with the formation of ceramide at the membranes of the endoplasmic reticulum (ER) (Figure 4). The condensation of the amino acid L-serine with a fatty acyl coenzyme A, usually palmitoyl coenzyme A, to 3-ketosphinganine is catalyzed by the enzyme serine palmitoyl transferase (SPT). This enzyme catalyses the rate-limiting step of this pathway. In most cell types, it preferentially utilizes fatty acid coenzyme A esters with a chain length of 16 carbon atoms resulting in long chain bases with a C18 chain. In a subsequent NADPH-dependent reaction, 3-ketosphinganine is reduced to D-*erythro*-sphinganine by the enzyme 3-ketosphinganine reductase. Sphinganine is acylated to dihydroceramide by the enzyme sphinganine N-acyltransferase. Dihydroceramide is substrate of a dihydroceramide desaturase leading to the formation of ceramide (82). The order of introduction of the double bond and acylation was controversial for some time, but it can be regarded as

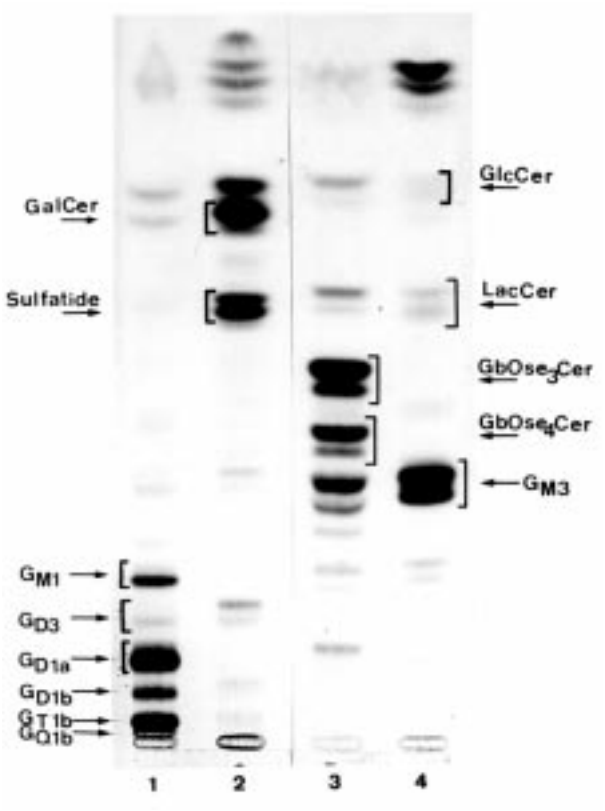

Figure 3. Biosynthetic labeling of cellular glycosphingolipids with [^{14}C]-Galactose (140). Cells were incubated for 48 h in the presence of [^{14}C]galactose (2 Ci / ml) and then harvested. Glycolipids were extracted, desalted, separated by thin layer chromatography and visualized by fluorography. Lane 1, primary cultured cerebellar neurons; lane 2, oligodendrocytes; lane 3, fibroblasts; lane 4, neuroblastoma cells (B104).The mobility of standard lipids is indicated. Abbreviations refer to the nomenclature of Svennerholm (133).

accepted that dihydroceramide is desaturated and not sphinganine (108). Therefore, sphingosine, the parent compound of the sphingolipids, is not an intermediate of sphingolipid biosynthesis but is formed during sphingolipid degradation. Besides sphinganine and sphingosine, another long chain base, phytosphingosine (D-ribo-4-hydroxy-sphinganine) is the structural constituent of many plant, yeast and mammalian epidermis sphingolipids (148).

Ceramide is the common precursor of GSLs and sphingomyelin. In the case of GSLs in vertebrates, a glucose or a galactose moiety is β-glycosidically linked to the 1-position of ceramide through the action of glycosyltransferases. The transferases utilize nucleotide activated sugars. Galactosylation of ceramide (126) takes place predominantly in oligodendrocytes and in

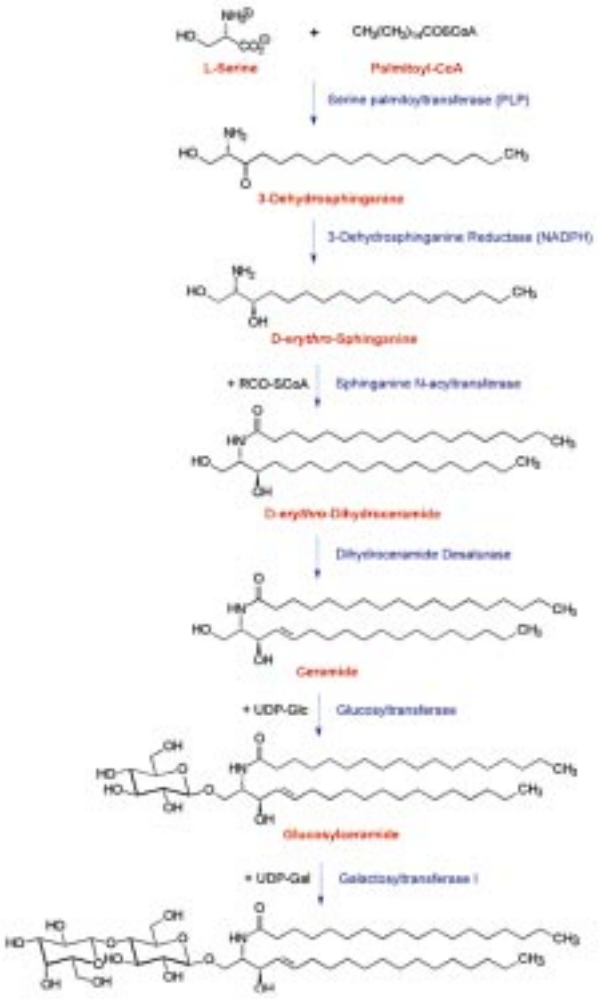

Figure 4. Biosynthesis of lactosylceramide (141). Heterogeneity within the lipid portion is not indicated.

the kidney. Galactosylceramide (GalCer) and sulfatide (GalCer-3-sulfate) are present in high concentrations in the multilamellar layer of myelin. The biosynthesis of most other GSLs of vertebrates requires the glucosylation of ceramide. The glucosylceramide synthase (50, 92) transfers a glucose residue from UDP-glucose to ceramide. Subsequently, lactosylceramide, the common precursor of the five GSL series found in vertebrates, is formed by the addition of a galactose moiety from UDP-Gal to glucosylceramide catalyzed by galactosyltransferase I (11).

Sphingomyelin biosynthesis requires the transfer of a phosphorylcholine headgroup from phosphatidylcholine to ceramide. Diacylglycerol is liberated in this step which suggests a tight coupling between sphingolipid and glycerolipid metabolism. Indeed, an inverse correlation between the amounts of sphingomyelin and phosphatidylcholine is observed in many membranes. The topology of this pathway (74, 142) is far from clear and contradicting or ambiguous results have been obtained in the investigation of its subcellular localization. However, the first three steps of sphingolipid biosynthesis leading to dihydroceramide are catalyzed by membrane bound enzymes at the cytosolic face of the endoplasmic reticulum (ER) (75). Since formation of glucosylceramide apparently occurs on the cytosolic face of the Golgi apparatus or a pre-Golgi compartment (142), dihydroceramide has to be transported from the ER to the Golgi apparatus, either by vesicle flow or by a protein-mediated process. Introduction of the next sugar residue leading to lactosylceramide appears to be restricted to the luminal site of the Golgi apparatus (65). This implicates a membrane translocation of glucosylceramide which is thought to be facilitated by a protein, a yet uncharacterized flippase. Biosynthesis of higher GSL proceeds on the luminal site of the Golgi apparatus (65). Therefore, the oligosaccharide chain of the GSLs bearing complex carbohydrates is orientated anticytosolic. This orientation is topologically equivalent to the situation in the plasma membrane, where the GSLs face the extracellular space. The bulk of sphingomyelin synthesis takes place on the luminal site (27) of an early Golgi- or pre-Golgi compartment although other sites for sphingomyelin synthesis have been reported. This requires an additional membrane translocation on the stage of ceramide. It is not clear whether this process is facilitated by a protein.

Ganglioside biosynthesis

The brain is particularly rich in sialic acid bearing GSLs of the ganglio series. Their biosynthesis (141) requires the sequential addition of carbohydrate residues including sialic acid to lactosylceramide by the action of membrane bound glycosyltransferases in the lumen of the Golgi apparatus. Lactosylceramide and its sialylated derivatives GM3, GD3 and GT3 serve as precursors for more complex gangliosides of the O, a, b and c series (Figure 5). C series gangliosides have been found only in trace amounts in human tissues. Sequential glycosylation of these precursors is performed by less specific glycosyltransferases, which transfer a respective sugar residue to glycosyl acceptors which differ only in the number of sialic acids bound to the inner galactose. In vitro data indicate that the sialyltransferases I and II are much more specific for their glycolipid substrates than sialyltransferases IV and V as well as the Gal- and GalNAc transferase. Many of the glycosyltranferases

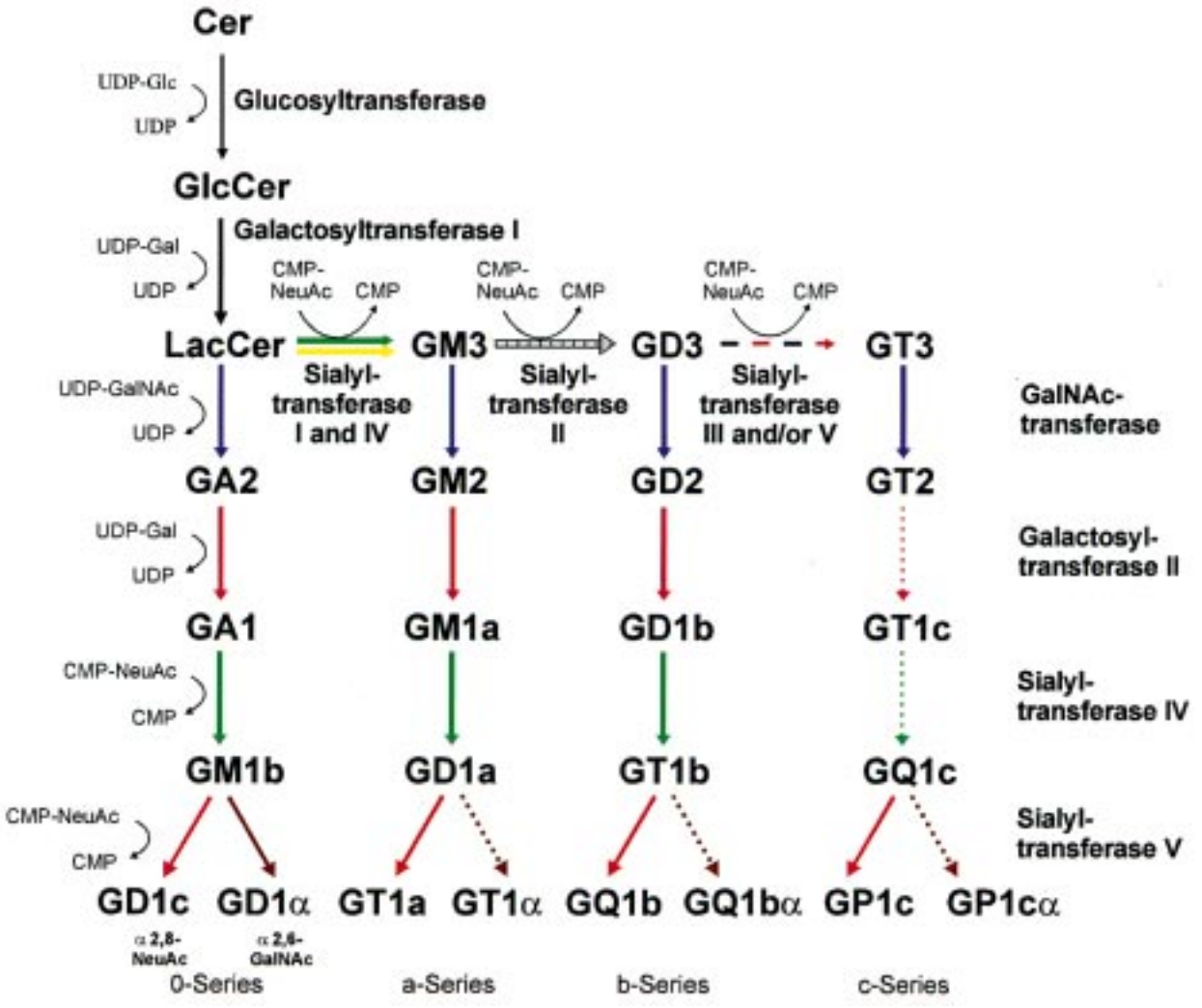

Figure 5. Ganglioside biosynthesis (141 considering 42). The reaction steps are catalyzed by membrane-bound glycosyltransferases in the lumen of the Golgi apparatus.

involved in GSL biosynthesis have been cloned in the recent past (138). Information on the distribution of glycosyltransferases within the stacks of the Golgi apparatus has been investigated with the aid of inhibitors of vesicular membrane flow. Besides de novo biosynthesis GSLs can also be formed in salvage pathways utilizing monosaccharides and sphingoid bases released in glycoconjugate catabolism (30).

Regulation

The maintenance of balanced GSL patterns on individual cell surfaces as well as on intracellular membranes requires a stringent control of GSL biosynthesis, degradation and intracellular traffic. The regulation of GSL metabolism and transport is not well understood and only a small amount of data is available (141). SPT seems to be the first control point for sphingolipid formation. The enzyme activity correlates with the relative amounts of sphingolipids found in different tissues. Sphingosine reduces the SPT activity in cultured neurons and removal of lipids from the skin leads to increased SPT activity (45). During ontogenesis and cell transformation a correlation between GSL expression and the activity of the glycosyltransferases leading to its synthesis has been observed. Therefore, transcriptional control of glycosyltransferases seems to be a major point of regulation. Since most glycosyltransferases have been cloned within the last few years (138), information required for the understanding of transcriptional control is expected to be available in the near future.

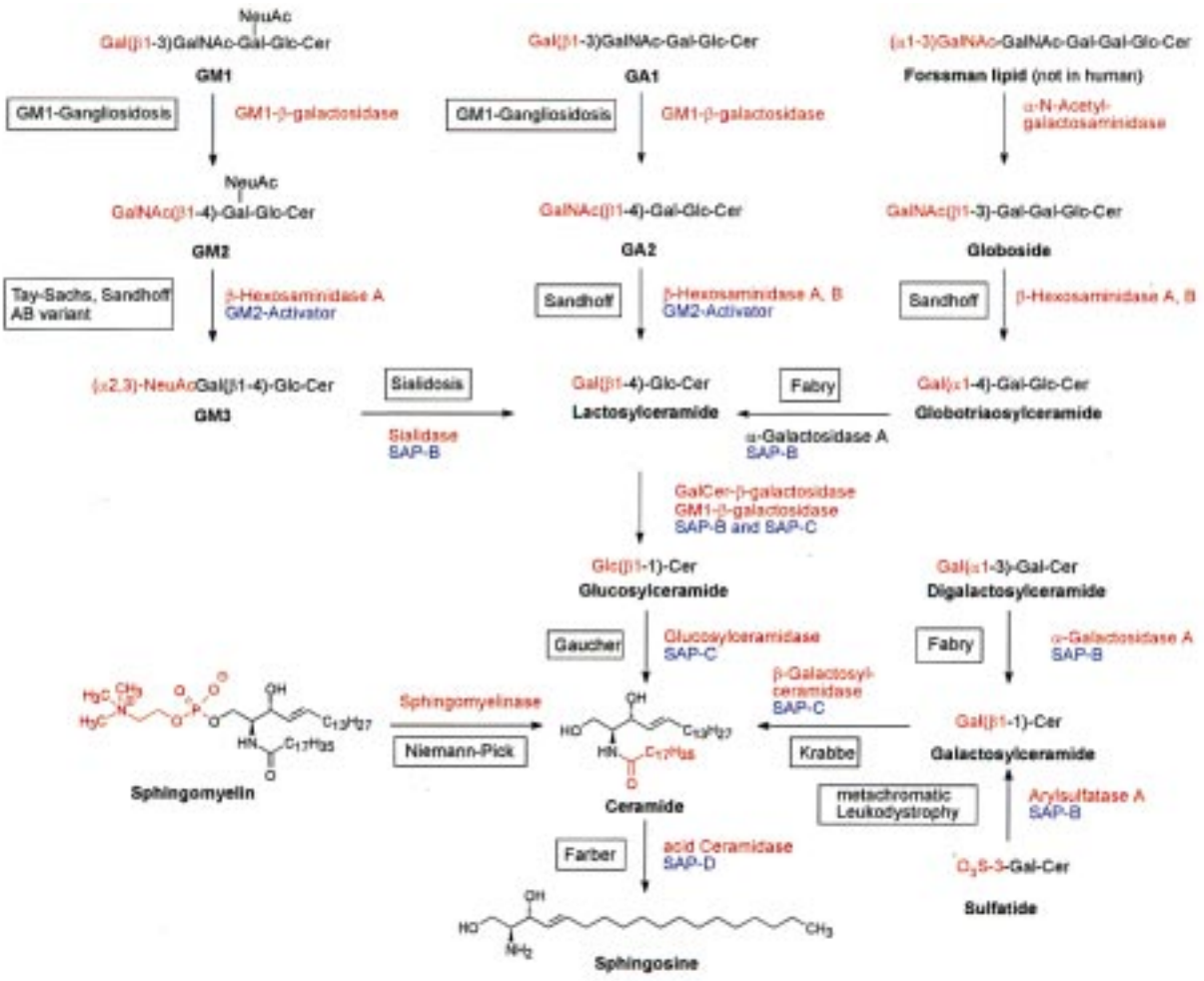

Figure 6. Degradation of selected sphingolipids in the lysosomes of the cells (59). The eponyms of individual inherited diseases (in frame) are given. Only those activator proteins which are required for the respective degradation step in vivo are indicated. Variant AB, AB variant of GM2 gangliosidosis (deficiency of GM2-activator protein); sap, sphingolipid-activator protein.

Besides regulation on the genomic level some findings hint on epigenetic regulation mechanisms. Feedback control of several glycosyltransferases either by its respective reaction product or final products within the corresponding series has been observed *in vitro*. Also the phosphorylation status of the glycosyltransferases can influence their activity. Lowering the pH of murine cerebellar cell culture media from 7.4 to 6.2 resulted in a reversible shift of ganglioside biosynthesis from the a- to the b- series. This observation can be explained by the complementary pH profiles of the key regulatory glycosyltransferases, sialyltransferase II and GalNAc transferase (49).

Sphingolipids in signal transduction

Lipophilic intermediates of sphingolipid catabolism have been identified as putative signaling molecules involved in the transmission of extracellular signals to intracellular regulatory systems (37, 128). In the case of the structurally related glycerolipids it has been well established for several years that extracellular agents are able to cause the formation or the release of lipid-derived second messengers like diacylglycerol, inositol-1,4,5-trisphosphate and others (21). Therefore, the function of phospholipids is not restricted to be structural constituents of the lipid bilayer of biological membranes. Also sphingosine, ceramide and their 1-phosphorylated derivatives are currently discussed as signaling molecules.

Increasing evidence suggests that ceramide plays a role comparable to its structural and functional glycerolipid counterpart, diacylglycerol (DAG). DAG,

together with inositol-1,4,5-trisphosphate, is released from phosphatidylinositol-4,5-bisphosphate by phospholipase C in response to an extracellular signal. The observation that sphingomyelin hydrolysis can also be induced by extracellular agents in various cell types, like lymphocytes, myelocytes or fibroblasts, led to the discovery of the so called sphingomyelin cycle. Tumor necrosis factor α, γ-interferon or interleukin-1, which act on receptors in the plasma membrane, and also calcitriol which acts on intracellular receptors, cause the formation of ceramide. The cellular and molecular effects of these extracellular agents, inhibition of cell growth, induction of differentiation, modulation of protein phosphorylation or regulation of gene transcription, are mimicked by application of a membrane-permeable ceramide derivative, C2-ceramide, which is the abbreviation used for N-acetyl-sphingosine. Importantly, the effects of C2-ceramide are generally not observed with the corresponding saturated derivative, C2-dihydroceramide (4). The identity of the cellular targets of ceramide and other molecules downstream within the signal flow is not unambiguously known. A ceramide-dependent kinase, a phosphatase and a protein kinase C subtype are currently under investigation (37, 55, 128).

Many effects have been reported to be mediated by ceramide. In general, ceramide appears to mediate antimitogenic effects like cell differentiation, cell cycle arrest and cell senescence. Several lines of evidence indicate ceramide to be a physiological mediator of apoptosis.

A large number of events are reported to be influenced by other intermediates of sphingolipid metabolism, e.g. inhibition of protein kinase C by sphingosine and lysoGSLs lacking the amide-bonded fatty acid (128). In these cases a clear coupling between extracellular receptor activation, intracellular elevation of sphingosine or sphingosine-1-phosphate and corresponding cellular responses is less evident compared to ceramide. Obviously, sphingosine-1-phosphate also operates as an autocrinic regulator in certain cell types, e.g. it is stored in platelets and can be extracellularly released to cause platelet aggregation (151). Other effects of sphingosine-1-phosphate in the blood (81) have been reported including activation of atrial myocyte potassium ion channels.

In contrast to ceramide, intracellular sphingosine-1-phosphate mediates mitogenic effects, e.g. it induces proliferation of Swiss 3T3 cells and stimulates the liberation of calcium ions from internal sources (128). Since ceramide, sphingosine and sphingosine-1-phosphate are metabolically coupled, it is not entirely clear which of these molecules is responsible for a distinct effect and why this pathway is mitogenic in some cells and antiproliferative in others. To date, a sphingolipid receptor involved in signal transduction has not unambiguously been characterized. It awaits elucidation how the cell regulates the function of these molecules either as metabolic intermediates or second messengers.

Glycosphingolipid degradation

The constitutive degradation of GSLs occurs in the acid compartments of the cells, the endosomes and the lysosomes. The plasma membrane containing GSLs destined for degradation are endocytosed and traffic through the endosomal compartments to reach the lysosome (114). The composition of sphingolipids entering the lysosomes depend on the cell type. Neuronal plasma membranes are rich in gangliosides while oligodendrocytes and Schwann cells have a higher content of galactosylceramide and sulfatide (Figure 3). Within the lysosome, hydrolyzing enzymes sequentially cleave off the sugar residues from the nonreducing end (Figure 6). Via lower glycosylated GSLs, ceramide and finally sphingosine are produced. This can leave the lysosome, reenter the biosynthetic pathway or be further degraded. More than ten different exohydrolases are involved in GSL degradation. If any of these enzymes is deficient, the corresponding lipid substrate accumulates and is stored in the lysosomal compartment. This leads to inherited lipid storage diseases with broad clinical and biochemical heterogeneity. Also enzyme substrates of other substance classes can accumulate in the lysosomes due to the specificity of the hydrolases for the residue to be removed and to a lesser extent for the backbone structure. For GSLs with long carbohydrate chains of more than four sugar residues the presence of an enzymatically active exohydrolase is sufficient for degradation *in vivo*. However, degradation of membrane bound GSLs with short oligosaccharide chains requires the cooperation of an exohydrolase and a protein cofactor, a so called sphingolipid activator protein (25). Several sphingolipid activator proteins are now known including the GM2 activator and the saposins SAP-A, -B, -C and -D. Inherited deficiencies of either lysosomal hydrolases or activator proteins give rise to GSL storage diseases (112).

Sphingolipid degradation is not necessarily restricted to occur in the lysosome. Sphingomyelin and ceramide can be cleaved by sphingomyelinases (127) and ceramidases (39) of various subcellular localization. Prior to degradation, sphingosines with the natural *erythro* configuration are phosphorylated by a sphingosine kinase

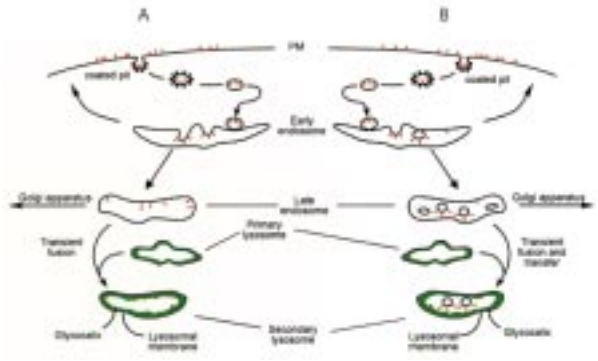

Figure 7. Two models for the topology of endocytosis and lysosomal digestion of glycosphingolipids (GSLs) derived from the plasma membrane (114). Conventional model (A): degradation of GSLs derived from the plasma membrane occurs selectively within the lysosomal membrane. Alternative model for the topology of endocytosis and digestion of GSLs (B): during endocytosis glycolipids of the plasma membrane become incorporated into the membranes of intraendosomal vesicles (multivesicular bodies). The vesicles are transferred into the lysosomal compartment when the late endosome fuses with primary lysosomes. PM, plasma membrane; •˙, (red) glycosphingolipid.

with cytosolic localization (8). The sphingosine-1-phosphate generated in this reaction can be cleaved by an enzyme localized on the cytosolic face of the endoplasmic reticulum, the sphingosine-1-phosphate lyase (143).

Lysosomal storage diseases

The degradation of cellular components occurs in the lysosomes. Macromolecules reach these organelles by endocytosis or autophagy and are cleaved by hydrolytic enzymes into their building blocks. Different hydrolases with acid pH optima are involved in this process, among them proteases, glycosidases, lipases, phospholipases, nucleases, phosphatases, and sulfatases. The degradation products leave the lysosomes and are used in other subcellular compartments for biosyntheses or energy production. Inheritable disorders within this degradation pathways lead to lysosomal storage diseases and are characterized by the accumulation of nondegradable enzyme substrates. They can be classified according to the stored substances, e.g. as sphingolipidoses, mucopolysaccharidoses, mucolipidoses, glycoprotein- and glycogen storage diseases (29, 87, 130). Not only the loss of a lysosomal hydrolase, but also the deficiency of transport- (28, 96) protective- (17, 18) and sphingolipid activator proteins (112) can cause a lysosomal storage disease. In addition to these protein deficiencies, incorrect posttranslational modification of lysosomal proteins can also lead to the expression of a lysosomal storage disease. This is the case in I-cell disease (Mucolipidosis II) (88) or in Austin's disease, a multiple sulfatase deficiency (56). In contrast e.g. to glycerolipid metabolism, defects in nearly every step in sphingolipid degradation are known to cause a human disease.

Topology of endocytosis and lysosomal digestion

Components of the plasma membrane (PM) reach the lysosomal compartment mainly by an endocytic membrane flow via the early and late endocytic reticulum (34) (Figure 7). During this vesicular flow lipids originating from the plasma membrane are subjected to a sorting process which directs some of the molecules to the lysosomal compartment, some others to the Golgi apparatus, and others back to the plasma membrane (79, 106). It remains an open question whether components of the plasma membrane reach the lysosomes as part of the lysosomal membrane. This is to be expected when the successive steps of vesicle budding and fusion along the endocytotic pathway are taken into account. It is, however, quite unlikely that portions of the lysosomal membrane originating from the plasma membrane can become selectively degraded by lysosomal enzymes without the destruction of the lysosomal membrane.

On the other hand, the observation of multivesicular bodies at the level of the early and late endosomal reticulum (114) suggests that parts of the endosomal membranes - obviously those enriched in components derived from the plasma membrane - bud off into the endosomal lumen and thus form intra-endosomal vesicles which become intralysosomal vesicles or other membrane structures by successive processes of membrane fission and fusion along the endocytic pathway. Therefore, glycoconjugates originating from the outer leaflet of the PM face the lysosol on the outer leaflet of intralysosomal vesicles. This hypothesis was introduced in 1992 (25) and is supported by a series of observations (114).

For example, multi-vesicular storage bodies accumulate in cells from patients with sphingolipid storage diseases. They were observed as early as in 1968 in cerebral cells from patients with GM1 gangliosidosis (131). Multivesicular storage bodies also occur in other cells, e.g., Kupffer cells and fibroblasts, of a patient with another sphingolipid storage disease, a combined activator protein deficiency (38, 121) Analysis of cultivated fibroblasts of this patient shows that the storage vesicles refer to late endosomal or lysosomal compartments. These compartments are still functionally active, except their inability to degrade sphingolipids with short oligosaccharide head groups. After complementation of

the medium of these cells with the missing SAP-precursor, not only the degradation block is abolished but also the morphological shape of the multivesicular bodies is reversed (9). Moreover, the epidermal growth factor receptor derived from the PM and internalized into lysosomes of hepatocytes is not integrated into the lysosomal membrane (105). The hypothesis is also in accordance with observations that spherical multi-vesicular bodies were the predominant endocytic compartments in HEp-2 cells and that multi-vesicular bodies matured within 60 to 90 min into lysosomes still containing internal vesicles (139).

Sphingolipid activator proteins

Degradation of GSLs occurs by stepwise action of specific acid exohydrolases. Several of these enzymes need the assistance of small glycoprotein cofactors, the so-called "sphingolipid activator proteins" (SAPs or saposins) (112) to attack membrane- or vesicle-bound GSL substrates with short oligosaccharide head groups. Since the discovery of sulfatide activator protein (77), other factors have been described but their identity, specificity and function have remained unclear until sequence data became available. It was found that the known activator proteins are encoded only by two genes (24, 86, 91). One gene carries the genetic information for the GM2-activator and the other for the SAP-precursor, which is processed to four homologous proteins including sulfatide activator protein (SAP-B) and glucosylceramidase activator protein (SAP-C) (145).

The GM2 activator

The *in vivo* degradation of ganglioside GM2, the major storage compound in the GM2 gangliosidoses, requires the enzyme β-hexosaminidase A and a lysosomal ganglioside binding protein, the GM2-activator (33, 112). A range of experimental data (78) suggests the mechanism of action of the GM2-activator. In contrast to the membrane-bound glycosyltransferases involved in GSL biosynthesis, β-hexosaminidase A is a water-soluble enzyme which acts on substrates of the membrane surface only if they extend far enough into the aqueous phase. Like a razor blade the enzyme recognizes and cleaves all substrates that stick out far enough into the aqueous space. However, GSL-substrates with oligosaccharide head groups too short to be reached by the water-soluble enzymes active site cannot be degraded. Their degradation requires a second component, the GM2-activator which binds ganglioside GM2 as well as structurally related gangliosides forming water-soluble complexes (usually in a 1:1 molar ratio). It complexes

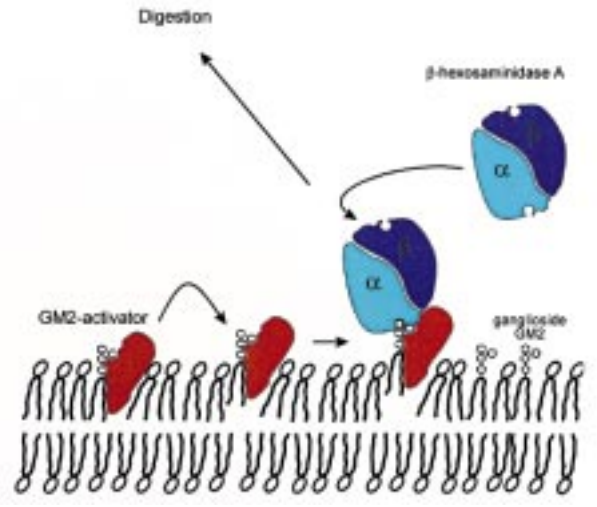

Figure 8. Model for the GM2-activator stimulated degradation of ganglioside GM2 by human b-hexosaminidase A (modified from 25). Water-soluble β-hexosaminidase A does not degrade membrane-bound ganglioside GM2, which has a short carbohydrate chain, in the absence of GM2-activator or appropriate detergents. The GM2-activator binds one molecule of ganglioside GM2 and lifts it out of the membrane. The activator-lipid complex can be recognized by water-soluble β-hexosaminidase A which cleaves the lipid substrate.

the substrate, lifting and eventually extracting it from the membrane and presenting it to the hexosaminidase A for degradation (Figure 8). Formation of the ternary complex presumably involves also a protein-protein interaction between the GM2-activator and β-hexosaminidase A (64).

Point mutations within the structural gene of the GM2-activator have been identified in four patients with the AB-variant of GM2-gangliosidosis (118, 123, 124) leading to premature degradation of the gene product. It is a common observation that altered processing of mutant proteins in the endoplasmic reticulum can contribute to the pathogenesis of genetic diseases (7). The resulting loss of the GM2-activator causes a block within the GM2 degradation in the lysosomes as demonstrated in metabolic studies in cultured fibroblasts of the patients. This block can be circumvented by feeding native GM2-activator to the culture medium of the mutant fibroblasts (53). Besides this only proven function of the GM2-activator in vivo, the lipid storage pattern observed in GM2-activator deficient mice indicates that it also facilitates the degradation of GA2 at least in this species (70).

In human epidermal keratinocytes, the GM2-activator is synthesized as a 22 kDa precursor and is trans-

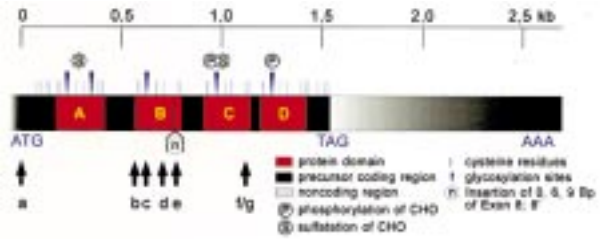

Figure 9. Structure of the SAP-precursor cDNA (25). The cDNA of SAP-precursor codes for a sequence of 524 amino acids (or of 527 amino acids, ref. 47) including a signal peptide of 16 amino acids. The four domains on the precursor, saposins A-D, correspond to the mature proteins found in human tissues: SAP-A is also known as saposin A; SAP-B is also known as saposin B or SAP-1 or sulfatide-activator; SAP-C is also known as SAP-2 or saposin C or glucosylceramidase activator protein; SAP-D is also known as saposin D or component C. The positions of the cysteine residues are marked by vertical bars and the positions of the N-glycosylation sites by arrow heads.
a. A1T (Met1Leu), (121). b. g-t-transversion within the 3' acceptor splice site between intron e and exon 6 (40). c. C650T (Thr217Ile), (104, 62). d. G722C (Cys241Ser), (47). e. 33 BP insertion after G777 (11 additional amino acids after Met259), (152, 153). f. G1154T (Cys385Phe), (122). g. T1155G (Cys385Gly), (103).

ported to the lysosomes in a mannose-6-phosphate-independent manner (31) which is in agreement with the observation that recombinant, nonglycosylated activator is efficiently endocytosed by fibroblasts from AB variant patients (53). Since lysosomal enzymes are thought to be transported in a mannose-6-phosphate-dependent manner (6, 60, 146) this constitutes a certain exception in the intracellular transport of a lysosomal protein. However, also targeting of the precursor of the other sphingolipid activator proteins, pSAP, from the Golgi apparatus to the lysosomes is only in part dependent on mannose-6-phosphate residues. Endocytosis occurs in a carbohydrate-independent manner since deglycosylated pSAP is taken up by mannose-6-phosphate receptor double knock out mouse fibroblasts more efficiently than the glycosylated protein (145).

SAP-precursor-derived activator proteins

The first activator protein was identified in 1964 as a protein which is necessary for the hydrolytic degradation of glycosphingolipids carrying a sulfuric ester group (sulfatides) by lysosomal arylsulfatase A (77). This activator, SAP-B (saposin B), is a small lysosomal glycoprotein consisting of 80 amino acids, with an N-linked carbohydrate chain and three disulfide bridges (26). Like the GM2-activator it binds GSLs, but with broader specificity so that it can be understood as a physiological detergent. *In vitro* it behaves similarly to the GM2-activator in some aspects, i.e. it can recognize and bind several different GSLs on the surface of micelles by forming a stoichiometric complex and is then able to transfer the GSLs to the membranes of acceptor liposomes (112). SAP-B can also present GSLs to water-soluble enzymes as substrates (69). The inherited deficiency of the sulfatide activator leads to a lysosomal storage disease which resembles metachromatic leukodystrophy. However unlike typical metachromatic leukodystrophy not only sulfatide but also additional glycolipids, e.g. globotriaosylceramide, accumulate owing to a blockage of degradation at several points in the catabolic pathway (119).

Together with three other activator proteins, (SAP-A, C and D) SAP-B is derived from a common precursor protein, pSAP (Figure 9). The four SAPs show homology to each other and have similar properties, but differ in their specificity and their mechanism of action. The physiological function of these sphingolipid activator proteins is only partially clarified to date; most of our knowledge on this has emerged from studies on patients with atypical lipid storage diseases. While the inherited deficiency of SAP-B leads to an atypical form of metachromatic leukodystrophy, SAP-C deficiency causes an atypical form of Gaucher's disease, where glucosylceramide accumulates (14, 122). In one patient with a complete deficiency of the whole SAP-precursor protein due to a homoallelic mutation within the start codon (121) there is simultaneous storage of many sphingolipids, including ceramide, glucosylceramide, lactosylceramide, ganglioside GM3, galactosylceramide, sulfatides, digalactosylceramide and globotriaosylceramide (5). Cells from these patients have been used to elucidate the in vivo function of SAP-D (52). When SAP-D is fed to the cells, ceramide accumulation is prevented. These studies on cells in culture from patients with different storage diseases support our hypothesis concerning the function of sphingolipid activator proteins. In their absence, membrane bound GSLs with short oligosaccharide chains are not readily accessible for digestion by water-soluble exohydrolases. By analogy to a razor blade, the hydrolases can only attack oligosaccharide chains of GSLs that protrude far enough from the membrane into the aqueous phase. This is no problem for long chain oligosaccharides but for the degradation of GSLs with short sugar chains the assistance of activator proteins is required. Some of them act as binding proteins or liftases, forming a water-soluble complex with the lipid and hence raising the lipid out of the membrane. However, the function of activator proteins is not necessarily restricted to binding lipids in this

way. For example, SAP-C can directly activate glucosylceramide b-glucosidase (43) and the GM2-activator specifically interacts with β-hexosaminidase A (64). Details of the intracellular targeting of pSAP are summarized in context with the GM2-activator (see above).

Pathogenesis of sphingolipidoses

The pathogenesis of sphingolipidoses is poorly understood although they are guided by few biochemical principles (113). On the one hand mutations in genes encoding different proteins can lead to diseases with similar or even identical phenotype. Thus, for example, deficiencies of the β-hexosaminidases α-chains, the β-chains or a deficiency of the GM2-activator protein give rise to similar clinical phenotypes that were characterized before as amaurotic idiocy (111). On the other hand mutations in the same structural gene can cause different clinical phenotypes. Even patients with identical mutations within the same structural gene e.g. the one encoding arylsulfatase A (93) can show different clinical forms, obviously due to a differing genetic background. Despite this enormous heterogeneity, and only indirect correlation between genotype and phenotype of the sphingolipidoses, it is possible to delineate some factors that connect genotype and phenotype in this group of diseases.

Due to the cell-type specific expression of GSL, lipid storage in lipidosis patients is observed especially in those cells and organs, in which the lipid substrates of the corresponding deficient enzyme are prevalently synthesized (e.g. complex gangliosides in neuronal cells) or by which they are taken up by phagocytosis (glucosylceramide and its precursors in macrophages in Gaucher's disease). The other decisive factor is the residual enzymatic activity of the gene product in the lysosomes (67). In general, a complete deficiency of a lysosomal enzyme leads to an early onset and a severe course of the disease. Many mutations, however, only lead to a partial loss of enzymatic activity. A residual activity of only a few per cent can be sufficient to delay the onset of the disease and cause a mild course of the disease. Only if the residual enzyme activity decreases below a critical value, an accumulation of nondegradable enzyme substrate is observed (threshold theory, see below).

A degradation disorder can be accompanied by the accumulation of toxic substances. This is especially the case in Krabbe's disease, where to some extent galactosylsphingosine (psychosine) accumulates in those cells, in which its is predominantly synthesized, the oligodendrocytes. This leads to destruction of these cells and to a severe demyelination (83). The absolute amounts of

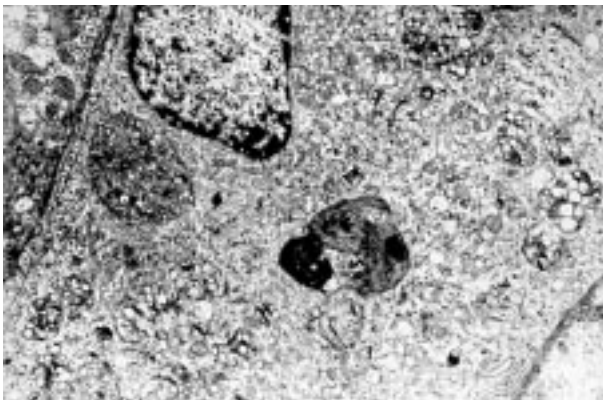

Figure 10. Liver biopsy of a patient with SAP-precursor deficiency. Sinusoidal cell with membranous inclusions, X 10,000 (38).

psychosine found in the white matter of the patients are very small, but this substance is usually undetectable in normal tissues and highly cytotoxic. Also other lysosphingolipids are thought to contribute to pathogenesis of sphingolipidoses like glucosylsphingosine in Gaucher's disease (89) or lysosulfatide in metachromatic leukodystrophy (137).

Finally, impeded degradation can cause the accumulation of morphologically active compounds (100, 102).

Threshold theory

Mutations in proteins of GSL degradation give rise to disorders in GSL turnover. Although having different effects on the corresponding proteins, they can be treated as v_{max} mutations that finally reduce the catabolic turnover of substrates (16).

In normal cells the maximum turnover rate of a catabolic system (v_{max}) is always higher than the influx rate (v_i) into the lysosomal compartment of the substrates to be degraded. Only if the maximum turnover rate becomes smaller than the influx rate, an irreversible substrate storage occurs and a storage disease develops. Due to mutations, the degrading system can achieve different residual activities which correlate with course and onset of the disease. Starting with the Michaelis-Menten equation, the steady state substrate concentration within the lysosome can be calculated as a function of the residual enzyme activity (16). In normal cells substrate concentrations are usually far below the Michaelis-Menten constant. A decrease of the maximum turnover rate of the degrading system on values of e.g. 50 to 20% of normal cells, a typical range for heterozygote carriers of inherited diseases, does not influence the turnover rate v. The ratio v/vi remains constant, since lowering of maximal enzyme activity is compensated by a higher

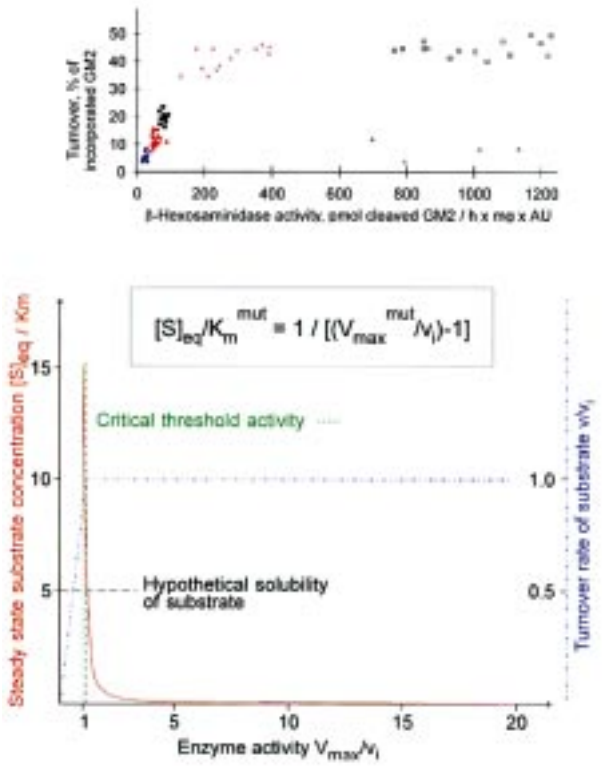

Figure 11. Theoretical correlation between the residual activity of an enzyme and the steady state concentration and turnover rate of its substrate in a defined compartment (16). The substrate concentration is expressed as multiples of K_m, turnover rate and enzyme activity (v_{max}) as multiples of the influx rate vi. Above the critical threshold activity ($v_{max} > v_i$), the turnover rate is limited by the influx rate; below this threshold ($v_{max} < v_i$) it is limited by the remaining capacity of the deficient enzyme; in the latter case, the substrate accumulates at a rate of $v_{an} = v_i - v_{max}$. —— (red), steady state substrate concentration; -.-..-.- (blue), turnover (flux) rate, ..., critical threshold activity; ---------- (green), critical threshold activity, taken the limited solubility of the substrate into account.

Above: residual activity of β-hexosaminidase A and turnover of its substrate ganglioside GM2 in cell culture. Skin fibroblasts from normal probands, from patients with the B variant and AB variant of GM2-gangliosidosis and from heterozygotes were fed with ganglioside GM2 for 3 days in culture. Cells were than harvested, homogenized in water and assayed for the following three parameters: incorporation of the substrate, percentage of degraded substrate, activity of β-hexosaminidase A towards ganglioside GM2 in presence of the GM2 activator protein.

Filled circles (blue): α-chain deficiency (B-variant of GM2 gangliosidosis, Tay-Sachs disease), infantile type; red circles: α-chain deficiency, juvenile type; filled squares: α-chain deficiency, adult type; green triangles: activator deficiency (AB-variant of GM2 gangliosidosis); healthy probands: x: obligate heterozygotes form GM2 gangliosidosis; empty squares: normal controls (from 113).

substrate saturation of the degrading enzyme (Figure 10). This compensatory mechanism works until the maximal turnover rate decreases to the value of substrate influx into the lysosome. At this critical threshold value all enzyme molecules occur in form of enzyme substrate complexes. The decrease of residual enzyme activities to this value should not lead to an irreversible pathological substrate storage. This explains that probands with pseudodeficiencies, e.g. a mean loss of enzyme activity of up to 90% of β-hexosaminidase A or arylsulfatase A are asymptomatic.

Only the decrease of the maximal catabolic enzyme activity (v_{max}) below the critical threshold value v_i , causes storage of the corresponding lipid substrate. The model predicts that the decrease of the maximal catabolic enzyme activity to the calculated threshold value does not influence the turnover rate of the substrate and that pathological storage occurs only below this level. This model was examined by measuring turnover rates and enzyme activities in cultured skin fibroblasts from patients with GM2 gangliosidoses (67). The experimentally observed values support the hypotheses since the turnover rate decreases linearly with decreasing enzyme activity only below a threshold value (Figure 11). Patients with an adult course of the disease show a significant higher turnover than patients with a juvenile course and these have a higher turnover than patients with an infantile course. Smaller differences in the residual enzyme activities correspond to significant differences in the respective turnover rates.

Animal models for metabolic studies

Animal models are a valuable means for the study of pathogenesis and approaches towards therapy of sphingolipid storage diseases. Besides naturally occurring animal models, mice models for several sphingolipidoses have been developed in the recent past (see Suzuki, Proia and Suzuki, this issue). In animal models of the GM2-gangliosidoses (59), differences in the sphingolipid degradation pathways between mice and humans have been discovered and a further physiological function of a group of degrading enzymes, the hexosaminidases, has been elucidated. Also an alternative approach towards therapy has been established in the animal model of one of these diseases (98). The GM2-gangliosidoses (33, 111) are a group of inherited disorders caused by intralysosomal accumulation of ganglioside GM2 and related glycolipids, particularly in neuronal cells. Three different genes code for the polypeptide chains involved in the degradation of ganglioside GM2: HEXA, which encodes the a-subunit of the het-

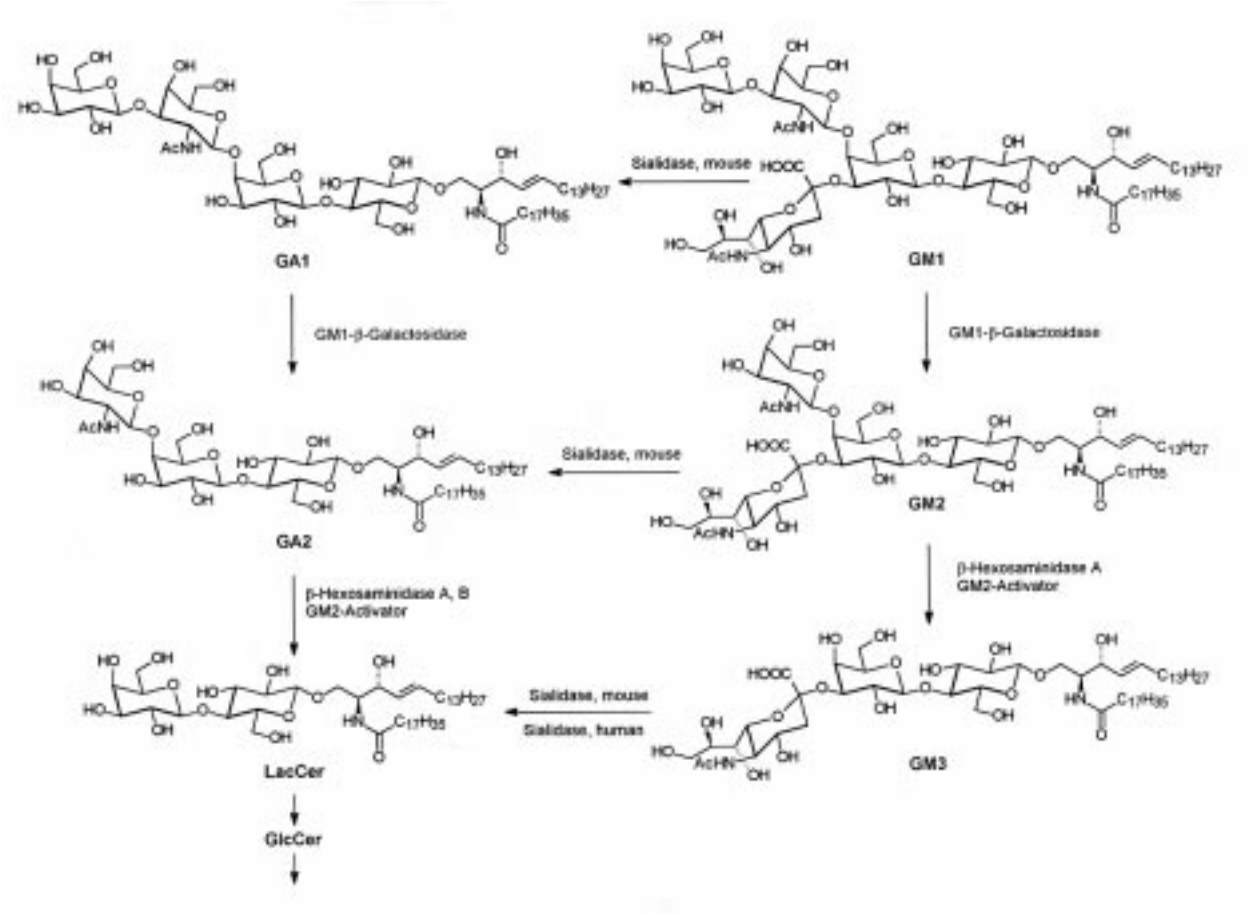

Figure 12. The ganglioside degradation pathway in mice and humans (116, 35).

erodimeric enzymes β-hexosaminidases A (αβ) and β-hexosaminidase S (αα); HEXB, which encodes the β subunit of β-hexosaminidase A (αβ) and β-hexosaminidase B (ββ); and GM2A, which encodes the GM2-activator. Three forms of the disease are distinguished: the B-variant with its infantile form usually called Tay-Sachs disease, resulting from mutations of the HEXA gene and associated with deficient activity of hexosaminidases A and S but normal hexosaminidase B; the 0-variant (Sandhoff's disease), resulting from mutations of the HEXB gene and associated with deficient activity of both β-hexosaminidases A and B; and GM2-activator deficiency (AB variant), due to mutation of the GM2A gene and characterized by normal hexosaminidases A and B. The gross pathology is very similar in B-variant, 0-variant, and GM2-activator deficiency, except that visceral organ involvement is evident in the 0-variant. The most pronounced cellular change is the presence of swollen neurons with massive accumulation of storage material in lysosomes throughout the nervous system. These form characteristic inclusions, the so-called membranous cytoplasmic bodies (MCBs). The hexosaminidases differ in their substrate specificity: β-hexosaminidase A cleaves off terminal β-glycosidically linked N-acetylglucosamine- and N-acetylgalactosamine residues from negatively charged and uncharged glycoconjugates. The natural substrate of b-hexosaminidase A is ganglioside GM2 which is negatively charged under physiological conditions. Uncharged substrates like the glycolipid GA2 can also be cleaved by b-hexosaminidase B.

The animal model of Tay-Sachs disease was generated by targeted disruption of the gene of the α-chains of β-hexosaminidase A in murine embryonic stem cells (15, 135, 150). The animal model of Sandhoff's disease has also been described, in which the β-chain of the hexosaminidases A and B are inactivated (94, 116). In the human phenotype, both variants of GM2-gangliosidoses mentioned above are only slightly different, but the animal models show drastic differences in severity

and course of the disease. Mice with deficient β-hexosaminidase A (the Tay-Sachs mice) are phenotypically normal. They show a mild accumulation of GM2-ganglioside in the central nervous system, but do not express the neurological symptoms characteristic for the human case of Tay-Sachs disease. In contrast, mice with deficient β-hexosaminidase B (Sandhoff mice) develop severe neurological disorders, the life span of the animals is severely reduced. The reason for this is the specificity of sialidase which is different between mouse and humans (116). Mouse sialidase accepts GM2-ganglioside as a substrate and converts it to GA2 (Figure 12). In the human, this metabolic pathway is of no significance since GA2 can be degraded by the still intact β-hexosaminidase B, so that in spite of a complete loss of β-hexosaminidase A the metabolic barrier is in part circumvented. Only the loss of both isoenzymes, hexosaminidases A and B, leads to the symptomatology corresponding to human Sandhoff disease. Although the mouse sialidase can still convert GM2 to GA2, GA2 cannot be further degraded, since the responsible enzyme, β-hexosaminidase B, is also deficient.

A double knock out mouse lacking the *hexa* and *hexb* genes has been developed without relation to a known human disease (115). These animals are deficient of all three isoenzymes, hexosaminidases A, B, and S. The brain of the double knockout mouse contained levels of GM2 and GA2 of a magnitude similar to the *hexb* -/- brain. Also notable was a decrease in the levels of cerebrosides and sulfatides in gray matter of the double knockout brain relative to wild-type, *hexa* -/- and *hexb* -/- mice indicating a severe demyelination. Besides accumulation of ganglioside GM2 these mice show a phenotype of mucopolysaccharidoses and excrete large amounts of glycosaminoglycans in the urine (115). Therefore, hexosaminidases seem to be responsible for the degradation of glycosaminoglycans. The mucopolysaccharidosis phenotype is neither seen in patients with GM2-gangliosidoses nor in the corresponding animal models. Here, the presence of at least one intact isoenzyme is sufficient to prevent storage of glycosaminoglycans and obviously there is a functional redundancy in the β-hexosaminidase enzyme system.

Pathobiochemistry of individual sphingolipidoses

The majority of enzymes and cofactors deficient in the sphingolipidoses have been characterized at the nucleic acid and the protein levels. Many mutations have been identified (29) and animal models of most sphingolipidoses have been created by targeted disruption of the respective genes in mice. The most recently purified and cloned enzymes are those which are deficient in Krabbe's disease and Farber's disease.

Krabbe's disease

Galactocerebrosidase, which is deficient in Krabbe's disease, is a membrane bound protein with a molecular weight of about 50 kDa (13). Galactocerebrosidase cDNA (12, 109) and the gene (73) have been cloned. Moreover, the cDNA of the murine enzyme has been cloned and the mutation responsible for the authentic mouse model for Krabbe's disease, the twitcher mouse, could be identified as a premature stop codon within the coding sequence (110).

Farber's disease

Acid ceramidase, the enzyme deficient in Farber's disease, has been purified from human urine. It is a heterodimeric enzyme composed of an α-subunit of 13 kDa and a β-subunit of 40 kDa (3). The cDNA encoding for both subunits has been cloned (54). The acid ceramidase needs the assistance of SAP-D for the *in vivo* degradation of ceramide (52). As it has been proven for most of the sphingolipidoses (67), the clinical course of the disease also correlates with the residual enzyme activity in Farber's disease (68).

Metachromatic leukodystrophy

A mouse model of metachromatic leukodystrophy (MLD) has been created that resembles the late infantile form of the human disease (41). It might become valuable for the investigation of therapeutic approaches and of the largely unknown early aspects of MLD pathology, e.g. the animal model shows involvement of the inner ear, where acoustic ganglion cells and myelinated nerve fibers were greatly reduced.

Austin's disease (mucosulfatidosis)

The molecular defect responsible for Austin's disease, a multiple sulfatase deficiency (MSD), could be identified as an erroneous posttranslational modification which appears to be necessary for sulfate ester hydrolysis. Many naturally occurring compounds contain hydroxyl groups which are chemically modified as sulfuric acid esters. They are cleaved by sulfatases of which nine have been characterized in humans and are localized in the lysosomes, with the exception of the steroid sulfatase (1, 88). In Austin's disease the activities of all known sulfatases are strongly reduced. The phenotype of the patients is clinically characterized as a combination of symptoms of metachromatic leukodystrophy and a mucopolysaccharidosis (56). The primary

defect in MSD remained unknown for a long time, although some observations pointed to a deficient co- or posttranslational modification (107). It could be shown that the posttranslational transformation of a cysteine into a formylglycine residue is deficient in cells from MSD patients (120). Obviously, this modification is required for the catalytic activity of the sulfatases.

B1-variant of GM2-gangliosidoses

The B1 variant of the GM2-gangliosidoses (63, 132) is distinguished from the B-variant (Tay-Sachs disease) by the altered substrate specificity of the mutated β-hexosaminidase A. Synthetic uncharged substrates that are used in the diagnosis are cleaved, while the natural substrate as well as the negatively charged synthetic substrates are resistant to degradation. It is assumed, that the active site on the α-chain of the β-hexosaminidase A is impaired without disturbance of subunit association or influence on the active site on the β-chain. Conclusions from mutations that an arginine residue should be involved in catalysis could be ruled out by x-ray crystallography of a closely related enzyme (22).

Niemann-Pick disease

In Niemann-Pick disease, type A and B, the acid sphingomyelinase is deficient. The enzyme has been purified (101), its cDNA (100) and genomic structure (125) analyzed and mutations leading to type A and B of the disease identified (29). The residual enzyme activity of type A patients is lower than that of type B patients (32) while the protein deficient in the nonallelic type C has only very recently been identified as a cholesterol homeostasis protein (10, 71). Since the identity of the sphingomyelinases involved in the signal transduction process through the sphingomyelin cycle is ambiguous, cells from Niemann-Pick patients were used to clarify this process. In contrast to control cells, lymphoblasts from Niemann-Pick patients and from the corresponding knockout mice showed no ceramide formation and no signs of apoptosis in response to irradiation (117). On the other hand, tumor necrosis factor alpha is able to activate NF-kappa-B in acid sphingomyelinase-deficient mouse embryonic fibroblasts (155). General conclusions on the identity of the sphingomyelinase involved in signal transduction cannot be drawn from the data available to date (136).

Acid sphingomyelinase shows a modular structure including a SAP-like domain. It is characterized by three intradomain disulfide linkages and several hydrophobic residues in presumably four α-helices. This structural motif is found in several lipid-binding proteins including the SAPs (84); its significance for the function of acid sphingomyelinase is not clear. In vitro results indicate that acid sphingomyelinase is stimulated by sphingolipid activator proteins, but this stimulation is not necessary for the *in vivo* degradation of sphingomyelin by this enzyme (5).

A further interesting finding, that treatment of animals with tricyclic antidepressive drugs, leads to symptoms similar to Niemann-Pick type B (72), has been explained by the induced proteolytic cleavage of the mature sphingomyelinase (48).

SAP-precursor

Some evidence indicates a function of the SAP-precursor of its own: the protein purified from human milk shows neurotrophic activity and protected hippocampal CA1 neurons from ischemic damage *in vivo*. This activity seemed to be mediated through an 18-amino-acid sequence located in the amino terminal hydrophilic region of the rat SAP-C domain (61). Moreover, it stimulated neuritogenesis and increased choline acetyltransferase activity in neuroblastoma cells (90). This effect was mediated by a 12-amino-acid peptide located in the amino terminal part of SAP-C, but does not overlap with the region responsible for the neurotrophic effect on hippocampal CA1 neurons. An animal model of SAP-precursor deficiency closely resembles the human disease (23).

Schindler disease

Schindler disease (20), an α-N-acetylgalactosaminidase deficiency, is the only inherited neuroaxonal dystrophy to date, in which the molecular defect is known. The nature of the storage material, however, is not unambiguously known. Pathological accumulation of enzyme substrates with terminal α-N-acetylgalactosamine residues could not been demonstrated.

Galactosialidosis

Galactosialidosis is characterized by the secondary deficiency of β-galactosidase and sialidase activity. The primary defect is due to mutations within the protective protein, which forms a stable complex with the GM1 β-galactosidase and the lysosomal sialidase (18). The protective protein shows also enzymatic activity as a serine esterase and a carboxypeptidase and a deamidase (17). Like activator protein deficiency, galactosialidosis represents a paradigm for substrate accumulation without mutations in the genes encoding hydrolytic enzymes. Eventually, also other lysosomal proteins might become labile in the absence of protective factors not character-

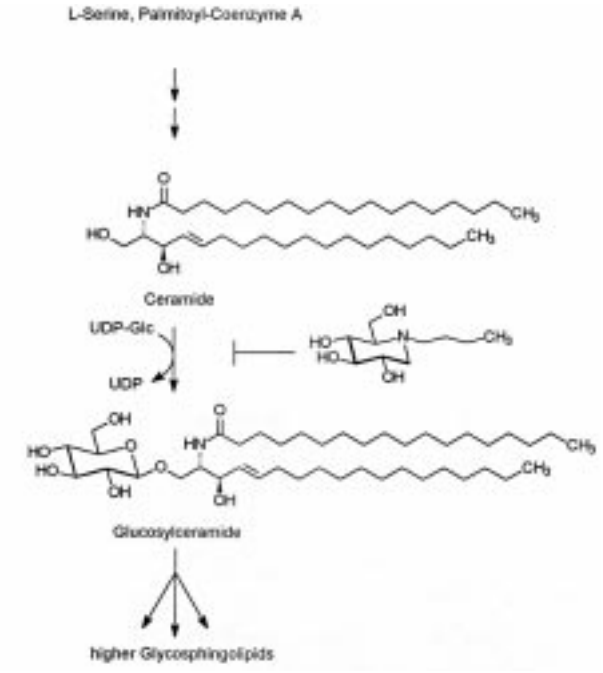

Figure 13. Part of sphingolipid biosynthesis and site of action of N-butyldeoxynojirimycin (from 57).

ized to date. A mouse model with defective protective protein turned out to be a phenocopy of the human disease, albeit with higher β-galactosidase activity (154).

Therapeutic approaches

Current strategies for the causal treatment of sphingolipidoses include enzyme replacement therapy, organ transplantation, and gene therapy. A causal therapy of sphingolipidoses is only available for patients of the adult form (type I) of Gaucher's disease (2). Here there is no involvement of the central nervous system due to the high residual activity of the deficient enzyme, the glucosylceramidase. To ensure correct targeting to the most affected macrophages, enzyme replacement therapy is carried out with a chemically modified glucocerebrosidase. To date, gene therapy-based approaches (144) and bone marrow transplantation has not lead to the desired success in the treatment of sphingolipidoses.

A further approach involving substrate deprivation has been reported and revealed promising results in the preclinical animal model of Tay-Sachs disease, the *hexa* -/- mice. A major factor that influences both, severity and course of sphingolipid storage diseases is the ratio of residual catabolic enzyme activity to substrate influx into the lysosome (16). As far as biosynthesis of enzyme substrates proceeds, the pathological accumulation of storage material continues. Substrate influx into the lysosome should be reduced by inhibition of sphingolipid biosynthesis. This approach requires a definite residual activity of the enzyme to be present in the lysosomes. This is the case in juvenile and adult patients. Recently, central nervous lipid storage in *hexa* -/- mice is retarded with the aid of a low molecular weight inhibitor of glucosylceramide formation (Figure 13), N-Butyldeoxynojirimycin (98). This compound has been known before as a glycosidase inhibitor and as an inhibitor of HIV replication *in vitro*, where it interferes with the posttranslational processing of viral glycoprotein N-glycosidically linked glycans. Treatment with the inhibitor by the oral route can maintain serum concentrations of 50 μM in the animals as well as in humans receiving this compound within a course of antiviral therapy. This concentration is sufficient to achieve a 50% reduction of GM2 storage after twelve weeks of treatment compared to untreated mice. In contrast to untreated mice, cytoplasmic membrane structures due to deposited lipid were hardly detected in the neurons of mice treated for sixteen weeks. The compound is well tolerated, passes the blood-brain barrier and reaches sufficient high concentrations in the nervous system to inhibit glucosylceramide formation in the desired extent. Application of the inhibitor to healthy mice results in GSL depletion of liver and lymphoid organs of the animals (99). A compound with improved selectivity compared to N-butyldeoxynojirimycin is the corresponding derivative with D-galacto-configuration (97). This compound, however, has not been investigated in the animal model.

Several other low molecular weight inhibitors of GSL biosynthesis are known (58) and may also become valuable tools for the treatment of gangliosidoses and related diseases. However, inhibition of biosynthesis upstream from glucosylceramide formation can be accompanied by immune suppression or the formation of toxic metabolites. Ceramide-based inhibitors of GlcCer formation might interfere with sphingomyelin formation or with ceramide-dependent signal transduction. In principle, inhibition of glucosylceramide formation should be suitable for the treatment of other sphingolipidoses where glycosphingolipids are stored that are biosynthetically derived from glucosylceramide and where a residual enzymatic activity is found in the lysosome. This is not the case in Krabbe's disease, metachromatic leukodystrophy, Farber's disease or Niemann-Pick disease. Of particularly importance is the ability of N-butyldeoxynojirimycin to pass the blood-brain barrier, its low toxicity and the possibility of oral treatment.

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