

Review

Sphingolipids: Critical players in Alzheimer's disease

Gerhild van Echten-Deckert^{a,*}, Jochen Walter^{b,*}^a Life and Medical Sciences (LIMES), Membrane Biology and Lipid Biochemistry Unit at the Kekulé-Institute, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany^b Department of Neurology, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

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ABSTRACT

Alzheimer's disease is characterized by the progressive accumulation of extracellular deposits of the amyloid β -peptide ($A\beta$) and intraneuronal aggregates of the microtubule associated protein tau. Strong genetic, biochemical and cell biological evidence indicates critical roles of $A\beta$ in the initiation of the pathogenic process, while tau might mediate its toxicity and neurodegeneration. $A\beta$ is generated by proteolytic processing of the amyloid precursor protein (APP) by β - and γ -secretases. Alternatively, APP can also be cleaved by α -secretase within the $A\beta$ domain, thereby precluding subsequent production of $A\beta$. APP and the three secretases are integral membrane proteins and follow secretory and endocytic trafficking pathways. Thus, the membrane lipid composition could play important roles in trafficking and metabolism of Alzheimer's disease related proteins.

Sphingolipids and especially complex gangliosides are abundant and characteristic components of neuronal membranes. Together with cholesterol, they confer unique characteristics to membrane domains, thereby regulating subcellular trafficking and signaling pathways. Thus, sphingolipids emerged to important modulators of biological processes including cell growth, differentiation, and senescence. Defects in sphingolipid catabolism are long known to cause severe lysosomal storage disorders, often characterized by neurological phenotypes. In recent studies it became evident that impaired sphingolipid metabolism could also be involved in Alzheimer's disease.

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Abbreviations: AD, Alzheimer's disease; $A\beta$, amyloid β -peptide; APP, amyloid precursor protein; apoE, apolipoproteinE; ASM, acid sphingomyelinase; BACE1, beta-site APP cleaving enzyme1; CerS, ceramide synthase; CTF, C-terminal fragment; EOAD, early onset Alzheimer's disease; ER, endoplasmic reticulum; DHcer, dihydroceramide; FAD, familial Alzheimer's disease; Ga β , GM1-ganglioside-bound- $A\beta$; GSL, glycosphingolipid; LOAD, late onset Alzheimer's disease; LSD, lysosomal storage disorder; NFT, neurofibrillary tangles; NMDA, N-Methyl-D-Aspartate; NPC, Niemann Pick disease type C; PHF, paired helical filaments; PS, presenilin; S1P, sphingosine 1-phosphate; SERCA, Sarcoplasmic/endoplasmic reticulum calcium ATPase; SK, sphingosine kinase; SL, sphingolipid; SM, sphingomyelin; SPT, serine palmitoyltransferase.

* Corresponding authors. Tel.: +49 228 732703; fax: +49 228 734845 (G. van Echten-Deckert), tel.: +49 228 28719782; fax: +49 228 28714387 (J. Walter).

E-mail addresses: g.echten.deckert@uni-bonn.de (G. van Echten-Deckert), jochen.walter@ukb.uni-bonn.de (J. Walter).

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1. Introduction

Alzheimer's disease (AD) is neuropathologically characterized by the accumulation of intraneuronal and extracellular protein aggregates, progressive synapse loss and neurodegeneration [1–3]. The intraneuronal aggregates called neurofibrillary tangles (NFT) consist of paired helical filaments (PHF) of the microtubule-associated protein tau, while extracellular deposits in form of plaques contain large amounts of aggregated amyloid β -peptides (A β). A β derives by proteolytic processing of the amyloid precursor protein (APP; Fig. 1). Genetics strongly support a critical role of A β in the pathogenesis of AD, because mutations are found in genes that encode proteins directly involved in A β generation [4,5]. The gene encoding APP was the first identified to contain mutations that cause familial forms of early onset AD (EOAD). Although APP is a large type I membrane protein of about 700–770 amino acids, mutations are only found within or close to the small A β domain and commonly affect the generation and/or aggregation of A β . Additional EOAD-causing mutations were identified in the two presenilin (PS) genes that encode the homologues PS1 and PS2 proteins. Interestingly, PS proteins represent the catalytically active components of the γ -secretase complex that is critically involved in the proteolytic generation of A β from APP [6–9]. However, mutations in the APP and PS genes are very rare, together accounting only for 1–5% of all AD cases. Most cases occur sporadically later in life (>65 years, late onset AD, LOAD). Thus, aging is the strongest known risk factor in AD. Nevertheless, genetic risk factors for LOAD have also been identified. By far the strongest association with LOAD shows the ϵ 4 allele of the apolipoproteinE (apoE) gene [10,11]. ApoE is a major lipoprotein in the brain mediating transport of cholesterol and other lipids between neurons and glial

cells [12,13]. The lipid transport function of apoE and the nature of APP and PSs as integral membrane proteins, indicate an important role of membrane lipid metabolism in the pathogenesis of AD.

Despite an extensive structural and functional variation, all neuronal cells exhibit a common morphological characteristic: a high cell surface to volume ratio due to specialized membrane extensions as dendrites and axons. The membranes are critical for characteristic neural functions, i.e. information flux via electrical signal propagation and synaptic transmission. J.L.W. Thudichum discovered in the 1880s the sphingolipids (SLs) while studying the chemical composition of the brain. SLs are ubiquitous components of biological membranes. Their glycosylated forms, the glycosphingolipids (GSLs), exhibit cell type specific profiles especially in the brain [14]. In neurons, sialic acid containing, complex GSLs called gangliosides are particularly abundant. In addition to the structural functions in cellular membranes, bioactive SLs such as ceramide and sphingosine 1-phosphate (S1P) play important roles in signal transduction [15]. Thus, it is not surprising that SL metabolism is closely related with various neurological disorders [16]. As part of lipid rafts, complex GSLs and sphingomyelin (SM) also confer unique characteristics to membranes [17]. Hence, interconversion of SLs determines membrane dynamics and trafficking [18,19]. Alterations of SLs have been observed in the brains of patients with different late onset neurodegenerative diseases and also in the aging brain [20–26].

Here, we will review the current knowledge and discuss the physiological and pathophysiological implications of SLs in AD, the most common neurodegenerative disorder. Of special interest is the question on whether and how age-dependent changes in SL metabolism might contribute to the etiology of AD.

2. Metabolic pathways of sphingolipids

All cells are able to synthesize SLs starting from common metabolites namely amino acids (usually serine) and activated fatty acids (usually palmitoyl-CoA, in the brain also stearoyl-CoA [27]). The condensation of these two compounds is localized at the cytosolic face of the endoplasmic reticulum (ER). This rate-limiting biosynthetic step is catalyzed by serine palmitoyltransferase (SPT, Fig. 2). The formed short-lived ketone intermediate, 3-ketosphinganine, is rapidly reduced to sphinganine, a reduction accompanied with the consumption of NADPH. Thus, the biosynthetic steps depend on the availability of reductive coenzymes, indicating that oxidative stress can affect formation of SLs. The formed saturated long chain base sphinganine is then *N*-acylated to form dihydroceramides (DHCer), a reaction catalyzed by a family of six ceramide synthases (CerS) which generate ceramides with distinct acyl chain length [28]. CerS1 synthesizing C18-ceramide is predominant in the central nervous system [29]. Note that CerS1 is particularly abundant in all neurons including those of the neocortex and the hippocampus, brain regions that are mainly affected by neurodegeneration such as AD [30]. Loss of CerS1 activity resulted in a reduction of ceramide formation and hence in a dramatic increase of free and phosphorylated sphingoid bases that were accompa-

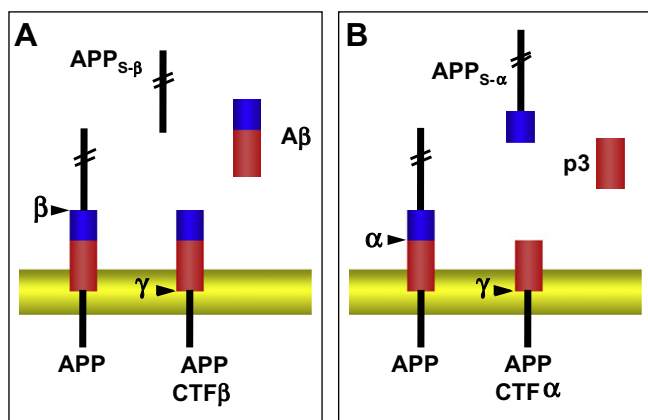


Fig. 1. Proteolytic processing of APP. APP is a type I membrane protein and can undergo two principal pathways in proteolytic processing. In the amyloidogenic route (A), APP is cleaved by β -secretase resulting in the generation of membrane-tethered CTF β and secretion of APP_{s- β} . The CTF β contains the full A β domain (blue–red box) and subsequent cleavage by γ -secretase liberates A β . Alternatively, APP can be cleaved by α -secretase to generate CTF α and APP_{s- α} (B). This cleavage occurs within A β domain thereby precluding the generation of A β . The subsequent cleavage of CTF α by γ -secretase results in secretion of peptide p3 (red box).

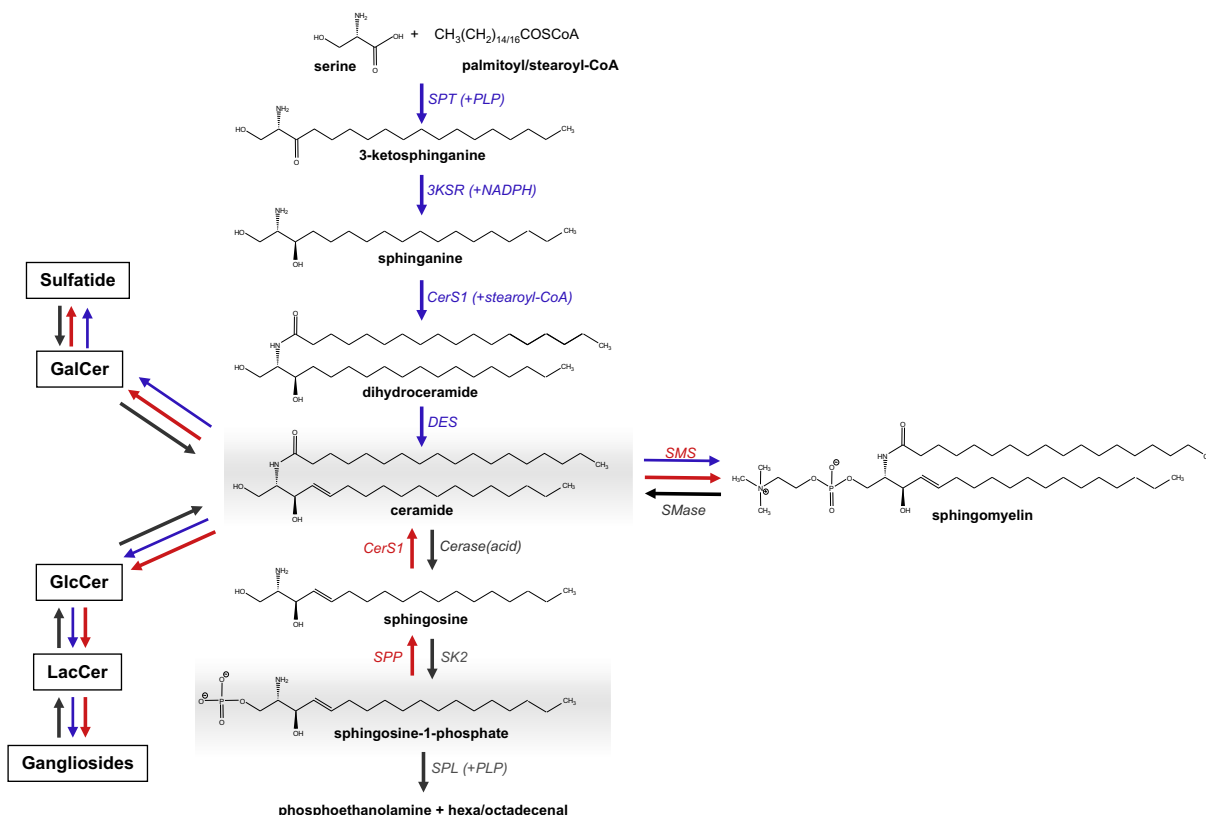


Fig. 2. Scheme of main sphingolipid metabolic pathways; Blue arrows, *de novo* biosynthesis; red arrows, recycling pathway; black arrows, degradation pathway. Abbreviations used are: i) for lipids: GalCer, galactosylceramide; GlcCer, glucosylceramide; LacCer, lactosylceramide; ii) for enzymes: SPT, serine palmitoyltransferase; 3KSR, 3-ketosphinganine reductase; CerS1, ceramide synthase1; DES, dihydroceramide desaturase; SMS, sphingomyelin synthase; SMase, sphingomyelinase; Cerase, ceramidase; SK2, sphingosine kinase2; SPL, sphingosine 1-phosphate lyase; SPP, sphingosine 1-phosphate phosphatase.

nied by Purkinje cell death and lipofuscin formation [31]. Desaturation of DHCer to yield ceramide is also NAD(P)H-dependent and catalyzed by DHCer-desaturase (DES) [32]. Ceramide, which anchors all SLs in the membrane bilayer was shown to mediate several biological functions in cells [33]. In addition it represents the main branching point in SL biosynthesis (Fig. 2). It is the direct precursor of phosphosphingolipids, including SM and of the GSLs, galactosylceramide (GalCer) and glucosylceramide (GlcCer), which can be converted stepwise into hundreds of complex GSLs, that are localized to the outer leaflet of the plasma membrane [34]. Sialic acid-containing gangliosides (Fig. 3) are enriched in neuronal membranes particularly in synapses [14,35], and are involved in fundamental neurobiological processes including neurodifferentiation, synaptogenesis and synaptic transmission [36,37]. Their potential role in the pathogenesis of AD has been extensively reviewed by Ariga et al. [38].

Metabolic homeostasis of the sphingolipidome in the brain is achieved by balancing of *de novo* biosynthesis, recycling, and degradation. The latter occurs mainly but not exclusively in the lysosomal compartment [14,39]. The carbohydrate head groups are cleaved stepwise yielding ceramide (Fig. 4). A family of ceramidases that act at different pH values and in different subcellular compartments hydrolyse ceramide to sphingosine and a fatty acid [40].

Notably, acid ceramidase is a major glycoprotein in human brain being mainly expressed in neurons [41]. The expression and activity of this enzyme were found to be elevated in AD brains and to co-localize with NFTs, thus suggesting an involvement in neuronal degeneration and hence in AD pathology [41]. Sphingosine can be phosphorylated by sphingosine kinases (SK) 1 or 2 to generate S1P. The two SK isoforms differ in kinetic properties,

subcellular localization and in developmental and tissue-specific expression [42–44]. In the rodent brain SK2 predominates and is most active in cerebellum followed by cortex and brainstem [45]. Also in human brain only SK2 was reliably detectable [46], suggesting that SK1 plays a minor role in the central nervous system.

S1P can either be irreversibly cleaved by S1P-lyase or dephosphorylated back to sphingosine by S1P phosphatase [14]. Of interest, S1P lyase and phosphatase are both localized in ER membranes. Sphingosine generated by S1P phosphatase at the ER can be rapidly recycled back to ceramide, which is then reutilized for the formation of SM and GSLs [47].

The expression and activity level of ceramidases, SK and S1P-lyase are critical for the regulation of bioactive SLs including ceramide, sphingosine and S1P that might be involved in neuronal survival.

3. Subcellular trafficking and metabolism of APP

The AD associated secretases as well as APP itself are integral membrane proteins that follow secretory and endocytic pathways. Thus, the localization and interaction of these proteins in different subcellular compartments determines the proteolytic processing of APP and generation of A β [48–50]. APP, α - and β -secretase are type I membrane proteins. In contrast, γ -secretase is a protein complex of four integral membrane proteins, called anterior pharynx-5 defective-1 (aph-1), nicastrin, presenilin enhancer-2 (pen-2), and presenilins (PS) 1 and 2. While aph-1, pen-2, and nicastrin appear to mainly function in the assembly, subcellular transport, and substrate recognition of γ -secretase, PS proteins represent the cata-

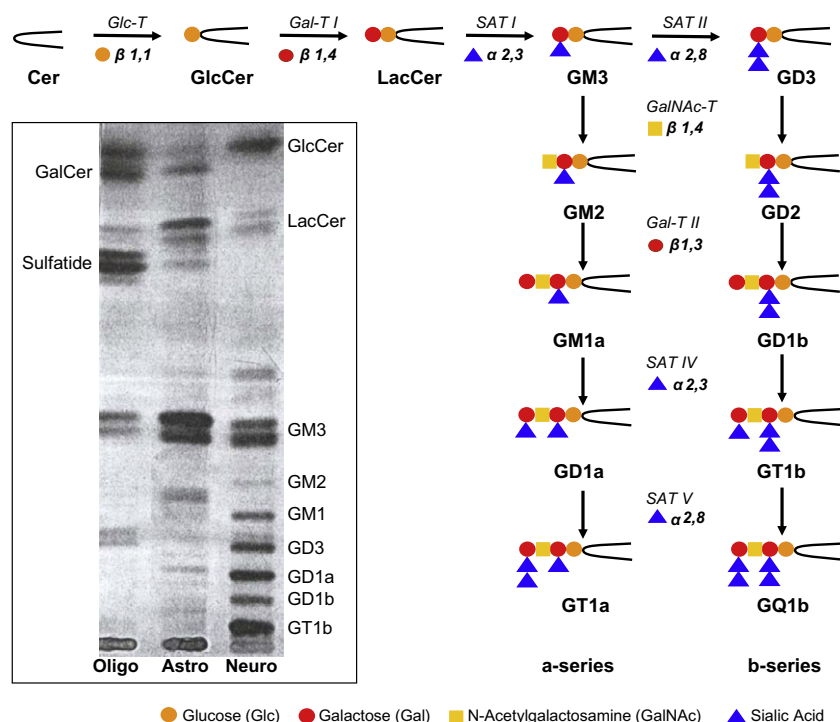


Fig. 3. Schematic depiction of major mammalian brain ganglioside biosynthesis. All glycosylation steps except the initial glucosylation of ceramide were mapped to the luminal face of Golgi membranes. GM3 and GD3, the precursors of a- and b-series of gangliosides, respectively, are formed by the action of specific sialyltransferases (SAT I and II) whereas rather unspecific glycosyltransferases catalyze the stepwise addition of a definite sugar as indicated. Inserted is a thin-layer-chromatogram showing cell type specific GSL profiles. Primary cell cultures were generated from rat brains and metabolically labeled with ^{14}C -galactose prior to extraction and separation of SLs. Abbreviations used are i) for lipids: Cer, Ceramide, N-Acylsphingosine; Gal, D-galactose; GalNAc, N-Acetyl-D-galactosamine; Glc, D-glucose; the terminology used for gangliosides is that of Svennerholm [267]; ii) for glycosyltransferases: GlcT, ceramide glucosyltransferase; GalT I, galactosyltransferase I (lactosyl synthase); GalT II, galactosyltransferase II (GM1a/GD1b synthase); GalNAcT, N-Acetyl-D-galactosaminyltransferase (GM2/GD2 synthase); SAT I, sialyltransferase I (GM3 synthase); SAT II, sialyltransferase II (GD3 41 synthase); SAT IV, sialyltransferase IV (GD1a/GT1b synthase); iii) for cell types: oligo, oligodendrocytes, astro, astrocytes; neuro, cerebellar neurons.

lytic subunits that mediate cleavage of APP and many other substrates [7,8,51,52]. After biosynthesis in the ER and maturation in the Golgi compartment, APP is transported in secretory vesicles to the plasma membrane. In the secretory pathway and at the cell surface, APP is predominantly cleaved by α -secretase, thereby precluding later generation of A β [50,53]. However, a fraction of full-length APP molecules can also undergo internalization into endosomal compartments. In these acidic vesicles, APP can be cleaved by β -secretase, an aspartic protease with an acidic pH optimum [53,54]. The resulting APP C-terminal fragment (CTF)- β (Fig. 5) represents the immediate substrate for γ -secretase. Thus, in endosomal compartments, or after recycling to the cell surface, APP CTF- β is cleaved by γ -secretase resulting in the generation and secretion of A β . It should be noted that the described subcellular sites of α -, β -, and γ -secretase dependent cleavage events for APP are not mutually exclusive, but rather represent predominant locations of the different processing steps. That β -secretase could also be active in the secretory pathway is well supported by findings with the Swedish mutation in APP, that causes EOAD. The double mutation at the β -secretase recognition site in APP strongly increases the recognition by β -secretase leading to more efficient cleavage already in secretory compartments, resulting in the direct competition with α -secretase and elevated secretion of A β [55–57].

In addition to secretory processing of APP by α -, β -, and γ -secretases, significant fractions of full-length APP and APP CTFs can be metabolized by other mechanisms, including proteasomal and lysosomal pathways. Accordingly, the inhibition of proteasomal or lysosomal activities led to stabilization of APP and potentially amyloidogenic CTFs [58–62]. This accumulation of APP CTFs might support A β generation by providing more substrate for γ -secretase.

The A β produced from APP CTF- β by γ -secretase in endocytic and lysosomal compartments might also be secreted upon recycling and fusion of these vesicles with the plasma membrane (Fig. 5). However, the intravesicular pool of A β and CTFs might also be degraded by endolysosomal hydrolases, as the inhibition of hydrolases or impairment of vesicular acidification led to accumulation of CTFs and intracellular A β [58]. Thus, the intravesicular pool of A β could contribute to the intracellular aggregates found in neurons of AD brains and APP transgenic mouse models [63,64]. However, extracellular A β and related aggregates could also be internalized and contribute to the intracellular A β deposits found in AD brains. Several mechanisms exist to clear A β from the brain. Microglia and astrocytes can internalize extracellular monomeric and aggregated forms of A β by endocytosis, pinocytosis and phagocytosis for further degradation in endosomal and lysosomal compartments [65–67]. Perivascular astrocytes are also involved in drainage of A β from the brain to the circulation via transcytosis [68,69]. Different cell types in the brain also express A β degrading enzymes, including proteases of the metallo-, serine-, aspartyl-, cysteine-proteases, that can contribute to the clearance of extracellular and intracellular A β pools [70,71]. The individual role of the various proteases and the different mechanisms contributing to A β clearance has been also described and discussed in excellent recent reviews [70–73].

4. Changes of sphingolipid pathways in the brain during aging and neurodegeneration – possible impact on AD

SL composition varies between different regions of the brain and characteristically changes during ontogenesis [35,74–77].

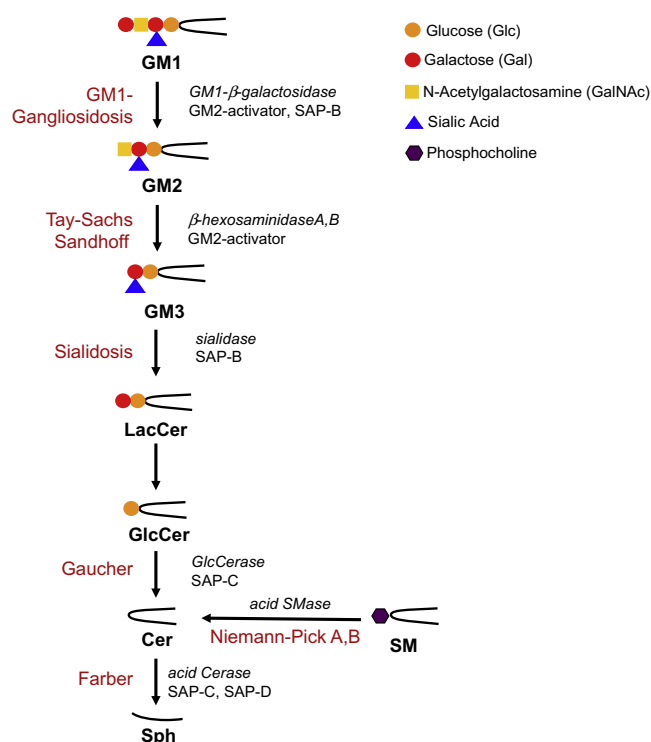


Fig. 4. Lysosomal degradation pathways of selected (glyco)sphingolipids affected in lipid storage diseases. Hydrolytic enzymes catalyzing SL degradation often need the assistance of an additional protein (GM2-activator or one of 3 saposins, as indicated). SM, sphingomyelin, Sph, sphingosine, all other abbreviations for lipids are as given in legend of Fig. 3. Cerase, ceramidase; GlcCerase, Glucosylceramide- β -glucosidase; SMase, sphingomyelinase; SAP, sphingolipid activator protein, saposin.

Fig. 3 reflects the specificity of GSL expression in different cell types of the brain. As compared to astrocytes and oligodendrocytes,

post-mitotic neurons express particularly high levels of complex gangliosides (Fig. 3).

When studying the literature on SL composition of the brain during development, aging and neurodegeneration, it is conspicuous that many studies concerning ganglioside composition of the human brain were conducted in the late 1980s using thin layer chromatography [35,78] or immuno-histochemistry [78,79], whereas more recent studies focused on less complex SLs like SM, hexosylceramides and predominantly on the signaling molecules ceramides and S1P [80]. Forty years ago scientists tried to understand the function of gangliosides and it was therefore important to know their distribution, while the signaling function of ceramide and S1P had not been identified at that time. In addition, determination of cellular lipids by more sensitive technologies based on mass spectrometry evolved especially during the last 10–15 years. Currently, electrospray ionization, tandem mass spectrometry (ESI-MS/MS) is used especially to determine less complex SLs in tissue or cell samples [81]. Similarly, multi-dimensional mass spectrometry-based shotgun lipidomics was employed to assess AD-related lipid pattern [82]. However, the determination of gangliosides with matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) proved to be quite tricky [83–85], which might explain the rather scarce number of mass spectrometry-based studies on brain gangliosides [86,87].

From the older studies it is evident that ganglioside content is highest in adult cerebral and cerebellar grey matter being 3-times higher than in cerebral and cerebellar white matter [35]. Ganglioside accretion is largest during the period of dendrite arborisation, axonal outgrowth and synaptogenesis [35]. While GM1a and GD1a, the two major a-series gangliosides (Fig. 3) of human brain markedly increase until the age of five and then slowly decrease with aging, GD1b and GT1b, the two main b-series gangliosides continuously increase until the age of 50 [35]. Between 20 and 70 years of age the total amount of gangliosides showed an almost constant concentration in the frontal and temporal cortices with a slight peak around 50 years of age [88].

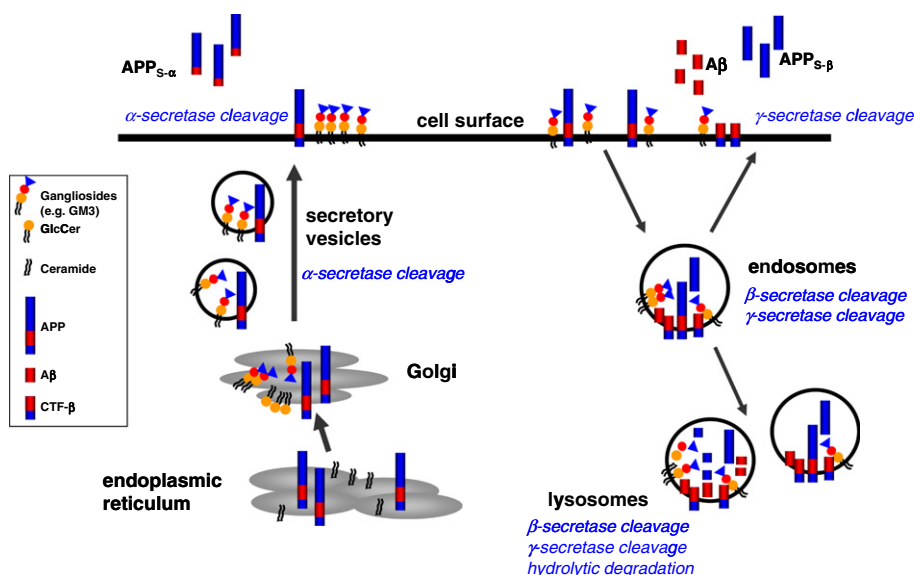


Fig. 5. Similar subcellular trafficking routes and metabolism of APP and GSLs. Biosynthesis of APP and GSLs starts in the ER. After transport to the Golgi, APP and GSLs are modified by glycosylation. On the way to and at the cell surface, APP is predominantly cleaved by α -secretase leading to the secretion of APP_{s- α} . Fractions of full-length APP that have not been cleaved by α -secretase can be internalized into endosomal compartments containing β -secretase and γ -secretase that process APP to CTF β and A β . After recycling of endosomes and fusion with the plasma membrane A β is released into the extracellular milieu. Note that γ -secretase is also present at the cell surface and could cleave APP CTFs, also resulting in secretion of A β . APP and its derivatives as well as GSLs can be transported from endosomes to lysosomes where major degradation occurs by lysosomal hydrolases. However, because β -secretase and γ -secretase are also present in lysosomes, these compartments can also contribute to A β generation. It is important to note that the described pathways represent the predominant processing events. Processing by β - and γ -secretases can also occur in secretory vesicles and the Golgi compartments and probably also in the ER.

Of note, certain ganglioside species containing C20-sphingosine (Fig. 2) occur exclusively in the nervous system [27], and their amount increases throughout life [89–91]. Using tissue-imaging mass spectrometry, the age-dependent accumulation of C20-ganglioside molecular species especially in the period of rapid synapse formation, dendritic outgrowth, and glial proliferation was visualized in murine hippocampus [87]. In addition, a distinct distribution of ganglioside species containing C18- or C20-sphingosine was established with this method. While the C18-species were widely distributed in the frontal brain, the C20-species selectively localized along the endorhinal-hippocampus projections especially in the molecular layer of the dentate gyrus [87]. To our knowledge no data concerning C20-gangliosides in AD brains are available so far.

A loss of gangliosides in AD was first demonstrated in 1965 in brain biopsies from three patients between 52 and 63 years of age [92]. Subsequent studies confirmed these findings but the numbers of samples determined were rather small and often incompletely defined as AD cases. The general interpretation of the results was neuronal loss with simultaneous demyelination. A series of studies performed in the late 1980s and early 1990s on ganglioside content and distribution in AD brains were less consistent [79,93–97]. There was general agreement on the reduction of total ganglioside levels in brain regions mainly affected in AD including temporal and frontal cortex, and nucleus basalis of Meynert. Further differences have been found in individual ganglioside species in AD brains as compared to controls. In some studies b-series gangliosides, GD1b and GT1b appear to be more affected by AD [94] whereas in others reduction of a-series ganglioside GD1a is more pronounced [97]. Most of the studies reported elevated concentrations of simple precursor gangliosides, GM3, GM2 and GD3 in AD brains [96,97]. This finding was explained by a higher degradation of complex gangliosides in AD brains. However, recent gene expression studies showing a reduced transcription of biosynthetic glycosyltransferases with no changes in the expression of catabolic hydrolases suggest a decreased formation rather than an increased degradation of GSL in AD brains [46]. In this regard it might be important to keep in mind that in post-mitotic terminally differentiated neurons gangliosides are synthesized predominantly from sphingoid bases recycled from the hydrolytic pathway [98]. Probably free sphingosine released from lysosomes is reutilized by ceramide synthases at the surface of the ER or in ER-associated membranes [99].

Sulfatides represent a class of sulfated galactosylceramides (GalCer) that are almost exclusively synthesized by oligodendrocytes (Fig. 3). In the central nervous system they occur mainly in the myelin sheath surrounding axons [100]. During aging their amount in the frontal and temporal white matter was reported to be reduced by about 35% between 20 and 100 years of age [88]. Mass spectrometric analysis demonstrated that sulfatide content was depleted up to 92% in gray matter and up to 58% in white matter of all examined brain regions in the earliest recognizable state of AD [101].

Sphingomyelin (SM) accounts for about 10% of mammalian cellular lipids. Although SM is a major lipid of myelin it cannot be considered to be a characteristic brain lipid as it also occurs abundantly in non-neural tissues. However, molecular species of SM, that contain stearic (C18) and lignoceric (C24) acids in their ceramide backbone were described to be more abundant in the brain as compared to other tissues [29]. Nevertheless, we found that in cerebellar neurons from 6-days-old mice SM (C16) was the predominant molecular species [98].

Several studies analyzed the content of SM in AD brains. The results obtained are, however, quite conflicting. Intriguingly, different methods yielded differing results. No differences between SM in AD and normal control brains could be detected with

electrospray ionization mass spectrometry (ESI-MS) [101]. Instead, an elevation of SM in several regions of AD brains and a positive correlation with the number of senile plaques was determined using ^{31}P -NMR [102]. By contrast, a significant reduction of SM in AD brains was reported recently [103]. However, the method used for quantification of SM in this study was quite complex. First, the lipid extract was subjected to enzymatic digestion with recombinant acid sphingomyelinase (rASM); the obtained ceramide was then subjected to enzymatic digestion with recombinant acid ceramidase. The formed sphingosine was chemically modified with NDA (naphthalene dialdehyde) yielding the respective fluorescent derivative, which was then separated by HPLC and finally quantified using a suitable calibration curve. Of interest, expression and activity levels of ASM were also determined and positively correlated with the levels of A β and hyper-phosphorylated tau protein in PHF-1 of AD brains [103]. Based on reports concerning stress-induced translocation of ASM to the plasma membrane and subsequent hydrolysis of SM and generation of ceramide-enriched platforms that mediate apoptotic cell death [104], one could conclude that ceramide generated by ASM might mediate A β and/or PHF-1 neurotoxicity. However, no positive correlation between A β , PHF-1, and ceramide could be identified [103]. Other studies document an involvement of neutral sphingomyelinase rather than of ASM in AD [105,106]. Yet, several studies reflect increasing ceramide levels in different regions of AD brains [22,101,103,107]. Interestingly, cellular senescence is also accompanied by ceramide enhancement [108]. Even though cell culture studies indicate a role of ceramide in neurodegeneration as well as in senescence [29,108–110], data on ceramide content in AD brains are still contradictory. Katsel et al. claimed that gene expression of enzymes controlling *de novo* formation of ceramide is gradually up-regulated while enzymes catalyzing glycosylation of ceramide are down-regulated especially in the temporal and frontal cortices of AD brains [46]. Paradoxically, the expression of SPT, catalyzing the rate-limiting step of *de novo* ceramide formation was rather unchanged or even reduced in early stages of AD [46]. Only CerS1 and CerS2 were significantly up-regulated whereas CerS6 was considerably down-regulated [46]. Together these data obtained with AD brains indicate a shift from C16 to C18 and longer ceramide species, which are generated from recycled sphingosine rather than *de novo* via SPT. In line with an elevated ceramide content in AD brains is, in addition, the significantly decreased gene expression of acid ceramidase reported in the same study [46]. Intriguingly, in a previous study both, expression and activity of acid ceramidase, were found to be considerably increased in the grey matter from fronto-temporal areas of AD-brains when compared with age-matched controls [41]. Similar results were obtained in a recent study, in which expression and activity of acid ceramidase were determined in membrane and soluble fractions prepared from AD brain samples [103]. These partially conflicting data demonstrate the importance of complementary analyses on gene and protein expression. Immuno-histochemical staining of acid ceramidase conducted in human brains showed no noticeable difference between AD and controls. Nevertheless, this staining indicated that acid ceramidase was located mainly in the cell bodies of neurons and co-localized with NFTs in AD [41]. However, no positive correlation between sphingosine, the enzymatic product of acid ceramidase and PHF-1 could be identified [103]. On the other hand, there was a negative correlation between the S1P content and A β or PHF-1 levels [103]. In this study the amounts of sphingosine and S1P were determined in the grey matter from fronto-temporal areas of AD-brains using an HPLC based method, in which sphingoid bases have to be chemically derivatized prior to separation. Note that increased amounts of sphingosine and decreased amounts of S1P were found only in the cytosolic fractions prepared from AD brains whereas their content in the

respective membrane fractions did not differ from normal controls. From the three enzymes involved in the sphingosine/S1P interconversion (Fig. 2), mRNA levels of SK2 and S1P phosphatase were not significantly different in AD brains when compared with controls. In contrast, mRNA of S1P-lyase was significantly elevated in AD brains [46]. However, in a recent study a clear up-regulation of SK2 enzymatic activity in AD brains was reported [111]. Moreover, this is the first description of a positive correlation between SK2 activity and the amyloidogenic processing of APP, caused by an S1P-induced activation of beta-site APP cleaving enzyme (BACE1). In line with these findings, S1P has been shown to be neurotoxic when generated by SK2 in lyase-deficient neurons [112]. Moreover, neuronal cell death was detected in mice lacking S1P-lyase activity [113]. Nevertheless, based on the fact that S1P counteracts ceramide-mediated apoptosis in many cell types [114] it has been suggested that S1P might be a strong neuroprotective factor against A β -induced neuronal apoptosis by inhibiting ASM activation [115]. It is important to note that a direct experimental evidence for an anti-apoptotic effect of S1P in post-mitotic, terminally differentiated neurons has not been provided so far.

The discrepancies between different studies on changes in SL metabolism in AD are remarkable. Although brain samples in most studies were taken from brain regions that are affected in AD, typically the fronto-temporal cortex and the hippocampus, variations might result from different post-mortem times and sample processing. Also, different methodological approaches could contribute to conflicting results as described in detail for SM determination (see above). In addition, it might be difficult to draw conclusions solely from gene transcription studies of enzymes involved in SL metabolism since SLs are structurally diverse and their metabolic pathways highly complex [116]. Our knowledge on post-transcriptional and post-translational regulation of these enzymes is still quite fragmentary. Also, the balance between formation, degradation and further usage as metabolic intermediates of certain SL species is complex and it appears unlikely to predict it only from enzyme transcript levels. However, a recent study describes a pathway visualization tool to predict differences in SL composition using microarray data [117]. There was a significant overlap between the gene expression data from two different cancer cell lines and the respective SL composition determined by mass spectrometry. It remains to be established whether this method is also applicable to the more complex ganglioside metabolism in nervous tissue.

In this regard caution is indicated when results obtained in diverse cell lines are transferred to post-mitotic terminally differentiated neurons. We recently demonstrated that SL and cholesterol metabolism in cultured neurons and brains, respectively, of S1P-lyase-deficient mice differs largely from that in liver or other tissues [98].

5. AD associated proteins and the metabolism of membrane lipids

The apoE is the major lipoprotein in the brain and mediates intercellular transport of cholesterol and other lipids [12,13]. In humans apoE exists in the three major isoforms E2, E3, and E4 that only differ in the combination of cysteine and arginine residues at positions 112 and 158. Interestingly, apoE4 that contains two arginines in these positions is by far the strongest risk factor for LOAD [118–120]. The isoforms differ in their conformation and stability that could influence the interaction with and transport of lipids in the brain. However, apoE knock-out mice show no gross change in brain cholesterol levels, and further work is required to understand the physiological role of apoE-dependent cholesterol metabolism in the brain and its pathophysiological implications for AD

[12,13]. Notably, apoE also binds A β and regulates its turnover. The deletion of apoE in APP transgenic mice decreases the deposition of A β and changes its overall distribution in different brain regions, indicating a direct effect of apoE on A β metabolism [121,122]. Although the exact mechanisms underlying these effects remain to be determined, recent data suggest a direct role of apoE in the aggregation of A β and its drainage from the brain via the blood brain barrier. The role of apoE in the cerebral metabolism of cholesterol and A β has been extensively discussed in recent reviews [12,13,123].

ApoE not only transports cholesterol and phospholipids, but also sulfatides. As mentioned above sulfatide levels have been found to be decreased in brains and in cerebrospinal fluid of AD patients and individuals with mild cognitive impairment [124]. Transgenic expression of the apoE4 isoform also led to significant decreases in sulfatide levels in different brain regions, while the E3 and E2 had smaller effects. It was suggested that astrocyte-derived apoE can bind and extract sulfatide from myelin and facilitate uptake and further degradation in other brain cells. Further examinations should reveal the pathways underlying the reduction in sulfatide in APP transgenic mice and pathophysiological consequences in AD. Notably, the FAD associated PS proteins also contribute to the regulation of cellular lipid metabolism. FAD mutations in PS1 increased biosynthesis of cholesterol and SM via A β dependent regulation of HMG-CoA reductase and sphingomyelinase [125]. Other studies also demonstrated increased levels of cholesterol in PS1 FAD mutant or PS knock-out cells, but proposed alternative molecular mechanisms involving altered endocytosis of apoE [126,127]. However, in all three studies impaired PS function commonly resulted in increased levels of membrane cholesterol. In addition, PS1 FAD mutations also impair the metabolism of phosphoinositides [128]. Together, these findings indicate a role of PS proteins and γ -secretase activity in the regulation of membrane lipids. It will be important to further dissect the molecular mechanisms that underlie the complex effects on different classes of lipids.

6. Sphingolipids in subcellular transport, proteolytic processing and degradation of AD associated proteins

APP as well as β - and γ -secretases are integral membrane proteins and thus tightly associate with membrane lipids [123,129–131]. Strong evidence indicates that the membrane lipid composition affects the subcellular trafficking and proteolytic processing of APP. The initial detection of A β together with full-length APP, APP CTFs, and PS1 in detergent-insoluble membrane microdomains, also called rafts, [132], stimulated a series of studies with cultured cells and animal models on the role of cholesterol in APP metabolism and A β generation [133]. Lowering membrane cholesterol by the inhibition of its biosynthesis with statins or extraction from cellular membranes with β -cyclodextrin could decrease A β secretion from cultured cells. However, a more subtle decrease in membrane cholesterol could also promote the secretion of A β [134]. While the underlying mechanisms remain to be determined in detail, the relative distribution of APP and secretase in rafts likely contributes to these effects [123,135–138]. Contrasting results in different studies might arise from the usage of different experimental models and procedures. The modulation of cholesterol esterification also affects the proteolytic processing of APP. The inhibition of Acyl-coenzyme A: cholesterol acyltransferase (ACAT1) led to decreased levels of secreted A β in cultured cells [139] and reduced plaque formation in APP transgenic mice [140]. However, the molecular mechanisms underlying the beneficial effects of ACAT1 inhibitors *in vivo*, remain to be identified, as no hints for altered α - or β -secretory cleavage of APP were found [140].

α -Secretase shows little association with cholesterol-rich microdomains and the non-amyloidogenic α -secretory processing appears to mainly occur outside of rafts. In contrast, BACE1 and components of the γ -secretase complex, including PS1 and PS2, aph-1, pen-2 and nicastrin showed higher association with rafts [141,142]. Accordingly, raft-associated fractions of APP might preferentially undergo amyloidogenic processing in these microdomains. It is interesting to note that APP CTF- β shows high association with biochemically isolated, Triton X-100 resistant membrane fractions, while only low amounts of full-length APP are found [132,141]. These data support a preferential generation of potentially amyloidogenic APP CTFs in cholesterol-rich microdomains. The localization of BACE1 to rafts depends on the palmitoylation of cysteine residues in its cytoplasmic domain [141]. Surprisingly, however, expression of palmitoylation-defective cysteine mutants of BACE1 did not alter A β generation in cultured cells, suggesting that BACE1 could cleave APP also outside of rafts, at least in overexpressing systems. Interestingly, specific targeting of BACE1 to lipid rafts by addition of a glycosyl-phosphatidyl inositol (GPI)-anchor significantly increased β -secretory processing of APP and A β generation [143].

Palmitoylation signals have also been identified in the γ -secretase components aph-1 and nicastrin. Although mutations of critical cysteine residues affected the stability of nascent aph-1 and nicastrin, the stability of mature γ -secretase complexes and association with rafts appeared not to be altered [141]. Thus, the molecular mechanisms regulating the association of secretases with membrane microdomains and the functional consequences related to APP processing endogenous expression levels remain to be identified. Interestingly, recent structural analysis by NMR spectroscopy and interaction studies indicated a specific binding of cholesterol to APP CTF β and probably the full-length APP as well [144]. This might have consequences for the distribution of APP and its CTF β in cholesterol-enriched domains and directly affect their processing by secretases.

In addition to cholesterol, SLs also affect the subcellular transport and the metabolism of AD-related proteins. The inhibition of ceramide biosynthesis decreased the generation of A β whereas the addition of a C6-ceramide or increasing endogenous ceramide levels by treatment with neutral sphingomyelinase elevated A β secretion in cultured cells [110]. This effect was attributed to a ceramide-mediated stabilization of BACE1. Further cell biological studies showed that lowering the levels of SM and GSLs decreased the maturation and forward transport of APP in the secretory pathway thereby reducing the fraction of this protein available for proteolytic processing by secretases [145–147]. In turn, addition of SLs, stabilized APP and increased the amounts of secreted APP variants and of A β [61,145,148]. However, mutant CHO cells with defective SL biosynthesis due to SPT deficiency showed increased production of A β 42, while A β 40 was not changed [147]. These contradictory findings might result from usage of different cell models and experimental conditions in the different studies. It is also important to note, that membrane lipid composition might affect the trafficking and proteolytic processing of APP and secretases expressed at the endogenous levels differently as compared to that of overexpressed proteins [145].

SLs can also modulate the enzymatic activity of secretases directly. In addition to the stabilization of BACE1 by ceramide in cells and the resulting increase in A β secretion [110], Kalvodova et al. demonstrated that certain lipids could directly stimulate the catalytic activity of BACE1 *in vitro* [149]. Surprisingly, the stimulatory effect was independent of the transmembrane domain of BACE1 suggesting that SLs could affect the conformation of the catalytic ectodomain and/or enzymatic activity of BACE1 allosterically. As mentioned in Section 4, S1P was also shown to increase the catalytic activity of BACE1. A direct interaction of S1P with the

transmembrane region of BACE1 was demonstrated in pull-down assays [111]. The reduction of S1P generation by pharmacological or genetic inhibition of SK decreased A β generation in neurons, but not in other cell types. Importantly, treatment of APP transgenic mice with SK inhibitors significantly reduced A β levels, suggesting a direct involvement of S1P in A β generation *in vivo* [111].

SLs also promote the activity of purified γ -secretase complexes reconstituted in phosphatidylcholine-based liposomes *in vitro* [150]. Further addition of cholesterol markedly increased γ -secretase activity, indicating that the complex composition of cellular membranes tightly regulates intramembraneous cleavage of APP as well as of other type I membrane proteins.

The ganglioside GM1 has also been shown to directly bind to the N-terminal domain of full-length and secreted APP and change its conformation. Because other SLs did not interact with the APP ectodomain, the glycomoiety of GM1 might determine this interaction. Thus, subcellular transport and proteolytic processing of APP might also be modulated by direct interaction with the head groups of SLs [151].

Apart from modulation of secretases involved in APP processing, SLs were also shown to affect the metabolism of APP independently of secretase activities. Enrichment of cellular membranes with exogenous SLs increased cellular levels of both, full-length APP and CTFs derived thereof. Interestingly, these effects were recapitulated in several primary fibroblast models of distinct lipid storage disorders (LSDs), including Niemann-Pick type A and B, Tay-Sachs and Sandhoff disease (Fig. 4) [60]. In line with this observation, accumulation of amyloidogenic APP CTFs was observed in different mouse models of LSDs, including Niemann-Pick type C (NPC), GM1 gangliosidosis, and Sandhoff disease [152,153].

Thus, the storage of different SLs induced similar phenotypes related to APP metabolism. An exception were fibroblasts from Farber disease patients that accumulate ceramide, but did not show accumulation of APP CTFs [60]. Cell biological studies revealed that the accumulation of APP and CTFs involves decreased clearance of these proteins in lysosomal compartments. In addition, increased SL levels could also stimulate autophagy induction [60,154]. Consistent with previous studies, increased induction of autophagy resulted in higher production of amyloidogenic APP CTFs. Thus, SL accumulation exerts dual effects on APP metabolism, namely increased production of amyloidogenic APP CTFs in autophagic vesicles and decreased lysosomal clearance [61].

7. Sphingolipids as modulators of protein aggregation

The etiology of a growing number of diseases appears to involve protein misfolding and aggregation [155]. Amyloid depositions not only occur in the brain, but also in other organs like liver and pancreatic islets [156,157]. Several factors were experimentally shown to favor protein aggregation. Apart from protein mutations and defects in the protein quality control systems such as molecular chaperones, and the ubiquitin proteasomal- as well as the autophagy-lysosomal-protein degradation [158,159], protein interactions with environmental factors have been considered. In the 1990s several studies suggested that A β -induced neurotoxicity in AD might be mediated by alterations of physicochemical properties of neuronal membranes as a consequence of A β interactions with these membranes. On the one hand A β was shown to reduce membrane fluidity in rodent brain [160], and on the other hand to selectively modulate membrane cation-permeability [161]. A crucial finding in support of an interaction between A β and neuronal membranes was the discovery of a unique ganglioside GM1-bound form of A β (GA β) [162]. A SL-binding domain in A β was reported later and is shared with prion and HIV-1 proteins [163]. Membrane-bound A β was hypothesized to act as a seed for amyloid fibrillogenesis [164]. Indeed, it could be

experimentally demonstrated that gangliosides are able to induce the conformational transition of A β from random coil and α -helical structure to a β -sheet-rich structure [165]. These experiments were conducted, in raft-like artificial membranes composed of cholesterol and SM. Notably, from the four major gangliosides of human brain, GM1 exhibited the strongest seeding potential. In grey matter of the frontal and temporal lobe, the amount of GM1 (14–16 μ mol/g) was shown to be less abundant compared to that of the other three major gangliosides (22–29 μ mol/g) whereas in the hippocampus GM1 (21 μ mol/g) appeared to be second most abundant following GD1a (39 μ mol/g) [97]. Moreover, in these brain regions GM1 levels were either unchanged or slightly increased in AD, while total gangliosides were reduced by 20–30% [97].

As depicted above (see Section 6) there is convincing evidence for a lipid-raft-dependent ability of A β to act as a seed for fibril formation [129]. In addition, the cholesterol-dependent sequestration of A β and a conformational change promoted by raft-associated GM1 were considered to be crucial for plaque generation [162,166,167]. Given the particular affinity between SM and cholesterol [168,169], it is not surprising that SM accumulation provides a favorable milieu for GM1-induced assembly of A β [170]. In contrast, selective hydrolysis of SM was shown to promote biogenesis of A β [110]. Notably, this study did not focus on lipid rafts as being essential for amyloidogenic processing of APP, but demonstrated that increased ceramide levels promote A β generation by stabilizing

β -secretase. This apparent contradiction raises the question on the relative contribution to amyloid generation and aggregation of ceramide increase on the one hand and of lipid rafts enriched in SM, cholesterol and GM1 on the other hand. Using a specific anti-GA β antibody the group of Yanagisawa performed a series of immunohistochemical studies that argue in favor of an essential role of raft-associated-gangliosides in the polymerization and hence deposition of A β in AD [171]. First, GA β could be detected in AD brains as well as in aged monkey brains [164]. Secondly, GA β formation could be correlated with presynaptic terminal-specific A β deposition, being favored by known AD risk factors like aging and expression of apoE4 [172,173]. Thirdly, accumulation of GA β occurred exclusively in subcellular structures of the endocytic but not of the secretory pathway [174]. Nevertheless, several experimental data argue in favor of additional SL-dependent molecular mechanisms that might be involved in the pathology of AD.

8. Common cytopathological changes in LSDs and AD

Although LSDs and AD differ in their genetic bases and age of onset, both diseases are characterized by largely overlapping phenotypes (Table 1).

Table 1

Overview of common cytopathological changes in Alzheimer's disease (AD) and lysosomal storage disorders (LSDs). Only references are cited that include studies with human cases and/or mouse models.

Phenotypes	AD	LSDs
Neurodegeneration	Yes [1]	Yes [268–270]
Neurofibrillary tangles	Yes	Yes
Hyperphosphorylated tau	[271,272]	[181,182, 273]
A β plaques	Yes	Not described
Accumulation of A β	Yes	Yes [152,153,179,180]
Accumulation of APP CTFs	Yes	Yes [152,153,179,273]
Accumulation of sphingolipids	Yes [80]	Yes
Endosomal/lysosomal pathology	Yes [196]	Yes [268]
Neuroinflammation	Yes [219]	Yes [218]
Impaired Ca ²⁺ signaling	Yes [274,275]	Yes [252,276]
Impaired autophagy	Yes [176]	Yes [276]

8.1. Decrease in lysosomal clearance capacity

Work from Dr. R. Nixon's laboratory demonstrated considerable alterations of the endosomal and lysosomal systems in AD [175,176]. Interestingly, increased number and size of endosomal/lysosomal compartments are one of the earliest cytopathological changes in AD and Down syndrome brains and appear to precede neurodegeneration. Moreover, lysosomal components can be found in extracellular plaques in association with A β . Likewise, levels of cathepsin D were shown to be increased in cerebrospinal fluid samples of AD patients as compared to controls, suggesting increased release of lysosomal hydrolases into extracellular fluids. Increased mRNA expression of cathepsin D was also observed in neurons of AD brains, indicating an upregulation of endosomal–lysosomal activity during AD pathogenesis, probably reflecting compensatory processes upon impairment of cellular clearance capacities [177]. The accumulation and enlargement of endosomal–lysosomal compartment that precedes neurodegeneration in AD is highly reminiscent to LSDs. Although the primary defect in NPC is related to cholesterol transport in endosomal–lysosomal compartments, SLs also accumulate in these compartments. Thus NPC might also be classified as a SL storage disorder. Indeed, a recent study indicated that elevation of cellular sphingosine levels correlated better with cytopathological changes related to calcium disturbance than cellular cholesterol levels [178]. Interestingly, in brains of NPC patients as well as in a cellular model of NPC, cathepsin D was redistributed to early endosomal vesicles, very similar to the localization of cathepsin D observed in AD brains [179].

In addition, brains of NPC patients also showed accumulation of amyloidogenic APP CTFs and A β 42 as compared to control brains [179]. However, A β 42 and APP CTFs appear to accumulate in rab5 positive early endosomal vesicles, rather than in rab7 positive late endosomal/lysosomal compartments. These changes were observed in a pharmacologically induced NPC model in primary mouse cortical neurons as well as in cerebellar Purkinje cells of human NPC brain. Accumulation of APP CTFs and A β was also shown in a mouse model of NPC. Analysis of human cerebrospinal fluid of NPC patients also revealed elevated levels of A β and tau [180]. Together, these data indicate that cytopathological changes characteristic for AD are also present in LSDs. It is also important to note that human NPC brains contain NFTs composed of tau very similar to human AD brains [181,182]. Interestingly, increased A β levels and tau-positive lesions were also observed in different human mucopolysaccharidoses [183], and a mouse model of mucopolysaccharidosis type IIIB [184]. Thus, classical neuropathological hallmarks of AD could also be induced by defective lysosomal activity. Recently, inhibition of lysosomal proteases was shown to induce altered lysosomal transport in cultured neurons [185], thereby resembling the accumulation of lysosomal compartments in axonal varicosities in neurons of the AD brain.

Interestingly, the PSs have been directly involved in lysosomal function [186,187]. Recent findings suggest that PS proteins support the targeting of v-ATPase to lysosomes thereby promoting lysosomal acidification. This function appears to be independent of γ -secretase activity and to involve direct interaction of full-length PS and the proton translocating v-ATPase V0a1-subunit in early secretory compartments [186,187]. FAD-associated mutations in PS resulted in an impaired transport of v-ATPase V0a1-subunits to lysosomes *in vitro*. Accordingly, lysosomal pathology was described in PS mutant mice and in human carriers [188]. These findings could also explain earlier reports on the accumulation and redistribution of other proteins including telencephalin and α -synuclein in PS deficient cells [189,190]. However, the proposed mechanism, PS-dependent targeting of the V0a1 subunit of the v-ATPase could not be confirmed in a subsequent study [191], although complex changes in genes involved in lysosomal biogenesis were observed in PS

knock-out cells and mouse brain. Together, the combined studies indicate that PS proteins and γ -secretase activity are involved in lysosomal function, but further work is required to understand the underlying molecular mechanisms.

8.2. Disturbed macroautophagy

Macroautophagy is a highly conserved process for the recycling of biomolecules, especially important during cellular adaptation under stress and starvation conditions [192–194]. This process is initiated by the formation of phagophores that derive from the ER and probably other compartments, including mitochondria [195]. Phagophores engulf cytoplasmic material, including aggregated proteins and mitochondria and seal to form autophagosomes. These double membrane vesicles can fuse with lysosomes thereby allowing access of lysosomal hydrolases to digest engulfed material. Autophagosomes can also fuse with late endosomal vesicles to amphisomes that finally fuse with lysosomes. Both, AD and LSDs are characterized by the accumulation of autophagic vesicles in somatodendritic and axonal compartments of neurons, which was initially anticipated to result from increased autophagic activity [196,197]. However, recent cell biological data assessing the autophagic flux indicated that rather decreased lysosomal clearance due to impaired vesicular transport and/or fusion might contribute to the increased number of autophagic vesicles [198,199]. Thus, the presence of enlarged endosomal and lysosomal compartments filled with cytoplasmic material more likely results from decreased lysosomal clearance capacity.

The importance of autophagy in neuronal homeostasis is indicated by *in vivo* studies with mice that have targeted deletions of autophagy-related genes in neurons. These mice overcome early death, but develop severe neurodegeneration, resembling the phenotypes of AD and LSDs [200,201]. Recently, it has been shown that activation of autophagy by rapamycin decreased the levels of intracellular A β of cultured cells and transgenic mice, suggesting that the stimulation of autophagy by the inhibition of mammalian target of rapamycin (mTOR) could be explored in AD therapy or prevention [202]. Importantly, rapamycin also could ameliorate cognitive deficits in APP transgenic mice [203]. However, activation of autophagy with rapamycin could also promote A β generation [60,204]. The mechanisms remain to be unraveled in more detail, but might involve increased sorting of APP and its CTFs together with secretases to similar autophagic vesicles [61].

Tau is also metabolized by autophagic pathways [205]. Cytosolic tau and proteolytic products can be degraded by macroautophagy [206], and thus, a decrease in autophagy could result in accumulation and further aggregation of tau [207]. Recent evidence also indicates that fractions of cytosolic tau could be proteolytically processed during chaperone-mediated autophagy. Upon association of tau with lysosomes via hsc70 and lamp-2a, tau is partially translocated to the lumen of lysosomes thereby allowing proteolysis by cathepsins. The resulting fragments are highly prone to aggregation [208]. Thus, while macroautophagy appears to be involved in the clearance of cytosolic tau, the partial processing of tau by chaperone-mediated autophagy promotes the generation of tau aggregates [209]. As these data are mainly derived from cellular models expressing truncated variants of tau, it will be interesting to characterize these processes also *in vivo*. The deletion of tau in a mouse model of NPC exacerbated the clinical phenotypes and led to earlier death [210]. These data could indicate that tau not only is metabolized via autophagic pathways, but also actively involved in their regulation, probably by modulation of vesicular transport.

Increasing evidence indicates a critical role of SLs in the regulation of autophagy [211,212]. Impairment of autophagy and lysosomal clearance capacity was observed in cellular and mouse models of different LSD, including NPC, GM1-gangliosidosis, and

mucopolisaccharidosis [213–215]. In a recent study *de novo* SL biosynthesis was reported to be essential for the formation of autophagosomes [216]. Also, increases of intracellular pools of S1P by depletion of S1P phosphohydrolase were reported to induce autophagy [217]. Thus SL metabolism and autophagic activity are functionally related. It would therefore be interesting to analyze whether age-dependent changes in SL metabolism impair the capacity of autophagic clearance and contribute to neurodegenerative processes.

8.3. Neuroinflammation

Another common phenotype observed in both, AD and LSD brains is neuroinflammation as indicated by the presence of GFAP-reactive astrocytes and activated microglia in human cases and mouse models of these diseases [218,219]. In AD brains, microglia appear to accumulate in close proximity to A β plaques and dystrophic neurites [219,220]. *In vivo* studies with APP transgenic mice demonstrate that A β deposits lead to activation and attraction of microglia followed by the occurrence of dystrophic neurites, suggesting that microglia could be involved in the process of neurite dystrophy [221]. The progressive neuroinflammation correlates with both, the accumulation of A β plaques and NFTs [222]. It is, however, still under debate whether inflammatory processes in the AD brain are beneficial or detrimental [219,223]. On the one hand microglia are capable to phagocytose fibrillar forms of A β via receptor-mediated internalization. Soluble A β variants could also be internalized by fluid phase pinocytosis and targeted to the endosomal/lysosomal system allowing degradation by several proteases, including neprilysin and cathepsins [224,225]. On the other hand, microglia secrete insulin degrading enzyme that cleaves soluble monomeric forms of A β [226–228]. The role of microglia in A β degradation, however, has been challenged in a recent study using APP transgenic mice with depleted microglia. No significant decrease in A β plaque load has been observed in brains of mice upon depletion of microglia [229]. As demonstrated by the progressive accumulation of A β deposits during AD pathogenesis, microglia obviously failed to efficiently remove A β from AD brains. The reason for this remains largely unclear. Pro-inflammatory cytokines could decrease the capacity of microglia to phagocytose A β in cell culture models [230]. Thus, due to the chronic inflammatory milieu in the AD brain, secretion of pro-inflammatory cytokines and other modulators of the immune system could impair A β clearance and also promote oxidative stress and neuronal damage. Brain inflammation has also been observed in LSDs. Microgliosis and astrogliosis were detected in mouse models of Sandhoff and Tay-Sachs disease that store ganglioside GM2, as well as in GM1 gangliosidosis and in NPC [218,231,232]. As demonstrated in mouse models of AD, LSD mice also showed increased brain expression of inflammatory surface proteins including CD11b, CD68 and MHC class II on microglia, and increased levels of TNF α and IL-6 β [218]. In addition, nitrotyrosine and iNOS were also detected indicating increased production of NO [218]. The occurrence of these factors strongly correlated with the extent of storage material and disease severity.

Interestingly, the inflammation and other disease symptoms in Sandhoff mice were attenuated by the pharmacological inhibition of GSL biosynthesis with *N*-butyldeoxynojirimycin (NB-DNJ) or bone marrow transplantation [218]. In a mouse model of Sandhoff disease bone marrow transplantation to substitute the defective enzyme, decreased the severity of lipid storage and also reduced brain inflammation [231]. While these data strongly indicate a critical role of accumulated lipids in driving brain inflammation, their exact roles need to be characterized in more detail. It has been shown that gangliosides could directly stimulate microglia activation and cytokine production in cell culture [233]. However, other

components of degenerated neurons might also contribute to the activation of microglia and the inflammatory process.

In addition, treatment of NPC mice with NSAIDs prolonged life span and delayed the onset and progression of clinical symptoms [234]. It is interesting that NSAIDs also showed protective effects against AD in epidemiologic studies [235–237].

8.4. Impairment in calcium signaling

Calcium deregulation also appears to contribute to the pathogenesis of AD as well as of LSDs. In AD, impairment of neuronal calcium homeostasis might be both cause and consequence of pathogenic events that contribute to the slow neurodegenerative process in sporadic AD. Already 25 years ago, a calcium hypothesis underlying brain aging and AD has been formulated [238], which still receives strong support by numerous studies that demonstrate a close relation of neuronal Ca^{2+} and AD associated proteins. On one hand sustained increased cytosolic Ca^{2+} concentrations can lead to elevated $\text{A}\beta$ generation [239]. On the other hand, exposure of cultured neurons to aggregated $\text{A}\beta$ induced elevation of cytosolic Ca^{2+} thereby increasing their susceptibility to excitotoxicity [240], likely involving direct disruption of membrane integrity by pore-forming conformations of $\text{A}\beta$ [241,242]. In addition, soluble $\text{A}\beta$ oligomers can also bind to NMDA receptors and increase their activity and excitability [243,244].

Interestingly, FAD causing mutations in PS1 and its homolog PS2 also induce increased release of Ca^{2+} from ER stores [245–247]. These effects could be attributed to the direct interaction of PS proteins with Ca^{2+} modulating proteins in ER membranes, including the smooth ER Ca^{2+} -ATPase (SERCA), IP3- and ryanodine-receptors [248–250].

PS proteins can also act as ER Ca^{2+} leak channels, thereby regulating the resting Ca^{2+} concentration in the ER in concert with SERCA pumps [251]. Most FAD mutations studied decrease the leak function of PS proteins and consequently lead to increased ER Ca^{2+} concentrations. However, the relative contribution of the impaired ER Ca^{2+} homeostasis caused by FAD associated PS mutations to AD pathogenesis needs to be determined. Calcium signaling appears to be also affected in LSDs. Increased cytosolic Ca^{2+} levels have been described in mouse models of GM2 gangliosidosis and Niemann-Pick type A, and were associated by decreased uptake into ER stores by SERCA [252–254]. In a neuronal culture model of Gaucher's disease by pharmacological inhibition of GlcCer glucosidase, Ca^{2+} release from the ER was increased and linked to accumulated GlcCer involving ryanodine receptors [255,256].

In addition to the ER, altered Ca^{2+} mobilization from the lysosome has also been implicated in NPC cellular and mouse models. The luminal Ca^{2+} concentration in lysosomes is about 500 fold higher than that in the cytosol [257,258]. A potent endogenous regulator of lysosomal calcium release is nicotinic acid adenine dinucleotide phosphate (NAADP) that targets two pore channels to the lysosomal membrane [259,260]. Recent data suggest that lysosomal sphingosine levels also contribute to the regulation of Ca^{2+} release from lysosomes. In a cellular model of NPC, sphingosine accumulates faster and to a higher extent than other SLs and cholesterol when normalized to basal levels [178]. Due to the acidic pH in lysosomes, sphingosine is protonated and thereby trapped in this compartment. The accumulation of sphingosine induces a decrease in lysosomal Ca^{2+} levels. While higher concentrations ($\sim 10\ \mu\text{M}$) of sphingosine might disrupt lysosomal membranes due to its detergent like properties and increase the luminal pH, lower sphingosine concentrations at levels observed in the NPC model ($\sim 1\ \mu\text{M}$) did not affect the lysosomal pH, indicating involvement of another mechanism in lysosomal Ca^{2+} release in NPC, which needs to be determined. The accumulation of lysosomal sphingosine and defective Ca^{2+} mobilization in NPC is

associated with aberrant endocytic trafficking further supporting a role of lysosomal Ca^{2+} mobilization in endocytosis and vesicular transport and fusion. Notably, Ca^{2+} mobilization from ER stores by cell treatment with thapsigargin partially restored proper trafficking of endosomal compartments.

We have recently shown that the neurotoxic effect of accumulating S1P also involves a disruption of the ER calcium homeostasis [113]. The signaling cascade triggered by elevated cytosolic calcium concentrations was mediated by calpain and involved CDK5 activation, hyperphosphorylation of tau, as well as aberrant reactivation of cell cycle events. Of note, a similar signaling cascade was described in an $\text{A}\beta$ -induced model of AD [261]. Although a growing number of studies document the close correlation of an impaired SL metabolism, calcium homeostasis and neuronal death a particular involvement of S1P in LSDs has not been analyzed so far.

8.5. Lysosomal lipid storage – cause or consequence of AD pathogenesis?

As detailed above, there is a strong similarity in the pathological features of LSDs and AD. While it will be difficult to dissect the sequence of pathogenic events in humans, the data obtained in cellular and animal models suggest that alterations of SL metabolism could be both, cause and consequence of protein accumulation and aggregation in lysosomal compartments. As the overexpression of APP or treatment of cells with $\text{A}\beta$ can induce alterations in the metabolism of membrane lipids and their accumulation in lysosomal compartments, it is evident that APP and/or its derivatives can drive lysosomal pathologies. In addition to the direct modulation of lipid metabolizing enzymes by APP and its derivatives, it has also been shown that endosomal and lysosomal compartments might be subcellular sites where $\text{A}\beta$ can form oligomeric or even higher order assemblies [262,263]. This build-up of aggregated material could further impair lysosomal integrity and function. E.g. a rupture of lysosomal membranes could impair the acidification of these organelles. The reduced activity of acidic hydrolases could then promote the accumulation of other biomolecules including membrane lipids. Further, the accumulated and aggregated proteins could sterically impair the interaction of lysosomal hydrolases with their substrates. Both mechanisms would

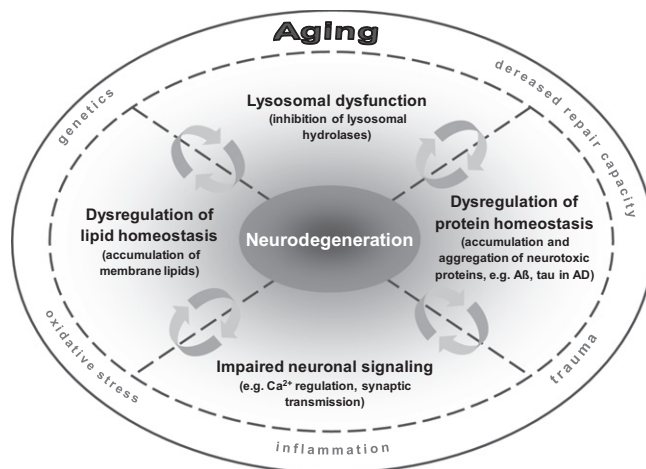


Fig. 6. Hypothetic sequence of age-dependent alterations leading to neurodegeneration. During aging, an increase in oxidative stress and decrease in repair mechanisms could lead to modification of enzymes and substrates impairing the lysosomal clearance capacity. The dysregulation of lysosomal activity causes further accumulation of lipids and proteins. It is important to note that accumulation of one could cause build-up of the other class of molecules. These processes affect neuronal signaling pathways and synaptic transmission ultimately leading to neurodegeneration.

eventually result in the accumulation of other biomolecules including membrane lipids.

On the other hand, the initial increase of membrane lipids as described above also can trigger accumulation of APP and potentially amyloidogenic fragments that favor formation of toxic A β aggregates. However, the relative contribution of both events to the pathological cascade in AD remains to be determined. It is likely that once an accumulation of either membrane lipids or aggregation-prone proteins occurred, the degradation of other molecules is also affected thereby driving a self-promoting cycle resulting in lysosomal dysfunction and increased susceptibility of neurons to degeneration (Fig. 6).

9. Conclusions and perspective

Aging has been termed a catabolic malfunction [264]. Increased levels of damaged macromolecules and organelles along with defective biological processes and increased oxidative stress characterize aged cells. One cytoprotective mechanism that prevents accumulation of harmful biomolecules and damaged organelles is autophagy. Diminution of this process during aging plays a major role in age-related accumulation of cellular waste [265]. As a consequence reactive oxygen species and the risk of mutations in DNA and defects in biological processes including lysosomal and autophagic functionality increase mainly in long-lived post-mitotic cells, such as neurons [265]. The particular abundance of gangliosides in neurons might explain why this cell type is highly sensitive to defective metabolism of these lipids in endosomal and lysosomal compartments. The decreased capacity to degrade gangliosides might reduce the availability of building blocks for recycling and thereby decrease the amount of gangliosides at synapses. However, more studies on the subcellular distribution of gangliosides are required to prove this assumption.

Based on our knowledge from LSD, it appears likely that an impaired endosomal–autophagic–lysosomal function also contributes to the neurodegeneration in AD and other age-dependent neurodegenerative diseases. However, in contrast to LSD, this process occurs much slower and over a long period during aging. Upon reaching a certain threshold, several detrimental processes could be initiated, including defects in calcium homeostasis, decreased clearance of amyloidogenic proteins, oxidative stress, hyperphosphorylation of tau, ultimately resulting in neuronal degeneration. When this cascade has already proceeded to massive loss of synapses and neuronal cell death, and has manifested in clinical symptoms, it might be difficult to reverse it, as indicated by the failure of recent clinical trials for AD therapy to improve clinical performance of AD patients [266]. As impaired endosomal–autophagic–lysosomal function affects primarily neurons that in contrast to other neural cell types express elevated amounts of complex gangliosides whose degradation requires several proteins and particular conditions [39], these stodgy components might constitute an initial risk factor for the neurodegenerative process. Thus, the early diagnosis and stabilization or activation of lysosomal lipid degradation could be valuable for future strategies in the treatment of AD and other late onset neurodegenerative disorders.

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