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Roles for Dysfunctional Sphingolipid Metabolism in Alzheimer's Disease Neuropathogenesis

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Abstract

Sphingolipids in the membranes of neurons play important roles in signal transduction, either by modulating the localization and activation of membrane-associated receptors or by acting as precursors of bioactive lipid mediators. Activation of cytokine and neurotrophic factor receptors coupled to sphingomyelinases results in the generation of ceramides and gangliosides, which in turn, modify the structural and functional plasticity of neurons. In aging and neurodegenerative conditions such as Alzheimer's disease (AD), there is increased membrane-associated oxidative stress and excessive production and accumulation of ceramides. Studies of brain tissue samples from human subjects, and of experimental models of the diseases, suggest that perturbed sphingomyelin metabolism is a pivotal event in the dysfunction and degeneration of neurons that occurs in AD and HIV dementia. Dietary and pharmacological interventions that target sphingolipid metabolism should be pursued for the prevention and treatment of neurodegenerative disorders.

Keywords

Alzheimer's Disease; sphingolipid; sphingomyelin; ceramide; sphingosine; ganglioside; synapse; amyloid

Introduction

Nearly two decades of research on roles for lipids in Alzheimer's disease (AD) suggest that a progressive disturbance in the composition of brain lipids may play important roles in the neuropathological process. These findings identify a disease-associated disruption in brain lipid biochemistry that deregulates levels of particular phospholipid, sphingomyelin, ceramide and ganglioside species in the brains of subjects with AD. Since a wide variety of signaling events are regulated by these lipids, a shift in the composition of lipids in brain cells would have profound effects on neural function. In this review we summarize our

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current state of knowledge for how disruptions in sphingolipid metabolism may promote aberrant amyloid processing, β -amyloid oligomerization and disruption of synaptic function in AD.

Overview of Sphingolipid Metabolism

Sphingolipid metabolism is complex and involves hundreds of molecular species and metabolic pathways. We do not attempt to fully address the complexities of sphingolipid metabolism in this section, rather it is intended to familiarize the reader with some relevant reactions involved in the metabolism of the major classes of sphingolipids (Figure 1). Knowledge of sphingolipid metabolism is essential when considering how perturbations in these pathways may contribute to aberrant amyloid processing and synaptic failure in AD.

Sphingolipids are derived from the aliphatic amino alcohol sphingosine. The sphingosine backbone is O-linked to a charged head group such as ethanolamine, serine or choline, and amide-linked to an acyl group, such as a fatty acid. *Ceramides* are the simplest sphingolipids, consisting of a fatty acid chain attached by an amide linkage to sphingosine. Ceramide is a precursor to *sphingomyelin*, which has a phosphorylcholine or phosphoethanolamine esterified to the 1-hydroxy group of ceramide. Ceramide can be deacylated to produce *sphingosine* that can then be phosphorylated to create *sphingosine 1-phosphate*. *Glycosphingolipids* are also derived from ceramides with the addition of one or more sugar residues, joined with a α -glycosidic linkage at the 1-hydroxyl position. Sphingolipids are enriched in the central nervous system (CNS), where in addition to important structural roles, sphingolipid metabolites function as second messengers to modulate a wide variety of signaling events. The major enzymes that regulate the metabolism of sphingolipids highlighted above are briefly discussed below.

Ceramide and Sphingomyelin

Ceramide is synthesized *de novo* in the endoplasmic reticulum with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase to produce 3-keto dihydrosphingosine. 3-keto dihydrosphingosine is then reduced to dihydrosphingosine by 3-keto dihydrosphingosine reductase. Dihydrosphingosine is N-acylated by ceramide synthase to create dihydroceramide. A final conversion of dihydroceramide to ceramide by dihydroceramide desaturase completes ceramide synthesis. In mammals, ceramide acyl chain length can vary from 16 to 26 carbons, depending on the particular ceramide synthase involved in their synthesis. There are 6 known ceramide synthases (CerS1-CerS6). These enzymes are also known as longevity assurance genes (LASS1-LASS6; see [1] for a review), with each mammalian enzyme utilizing a relatively restricted subset of fatty acyl-CoAs. The preferred fatty acid substrates are as follows: CerS1:C18, CerS2:C20-C26, CerS3:C18 & C24, CerS4:C18 & C20, CerS5:C16 and CerS6:C14 & C16. It is currently assumed that the six known mammalian CerS account for the synthesis of all known ceramides, but it is possible that other proteins, such as sphingomyelinases and ceramidases contribute to the synthesis of ceramides with restricted fatty acid composition.

Ceramide can also be created by the catabolism of sphingomyelin via a family of sphingomyelinases that hydrolyze the phosphodiester bond of sphingomyelin to create phosphocholine and ceramide. Sphingomyelinases are categorized based on optimal pH for activity into acidic- (aSMase), alkaline- (alkSMase) and neutral (nSMase). aSMase are Zn^{2+} dependent and primarily located to the lysosomal compartment [2], although there is also a secreted form of aSMase [3]. The alkSMase enzyme shares no structural similarity with other SMases (it belongs to the nucleotide pyrophosphatase/phosphodiesterase family), but does possess enzymatic properties similar to other sphingomyelinases [4]. Alkaline sphingomyelinase is located in the intestinal mucosa and bile where it functions in the

conversion of dietary sphingomyelin (see [5] for a review). There are currently three known nSMases that differ in subcellular location and ion dependence. nSMase1 is Mg^{2+} dependent and located to the endoplasmic reticulum [6]. nSMase2 is located in the Golgi apparatus [6,7], and this nSMase can also translocate to perinuclear regions in response to the antioxidant glutathione and to the plasmamembrane in response to oxidative stress [8-10]. nSMase 3 is located to in the golgi apparatus, endoplasmic reticulum and plasma membrane [11].

Ceramide can be converted to sphingomyelin by the transfer of a phosphocholine head group from phosphatidylcholine (Glycerophosphocholine) onto ceramide by the enzyme phosphatidylcholine transferase (sphingomyelin synthase). Currently, two sphingomyelin synthases designated as 1 and 2 (SMS1, SMS2) have been identified. These enzymes play critical roles in sphingolipid metabolism by catalyzing the conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol (DAG). Human SMS1 is localized to the Golgi, while SMS2 resides primarily at the plasma membrane [12-15].

Sphingosine and Sphingosine 1-Phosphate

Ceramide can be converted into sphingosine and a fatty acid by ceramidases. Sphingosine can be further converted to an anti-apoptotic lipid, sphingosine 1-phosphate by the enzyme sphingosine kinase. Similar to the sphingomyelins, ceramidases are categorized based on pH optima and cellular localization. There are 5 known human ceramidases that include acid ceramidase localized to lysosomal compartments, neutral ceramidase that localizes largely to the plasma membrane, an alkaline ceramidases 1, 2 and 3 which localize to the golgi apparatus and the plasma membrane (see [16] for a recent review of ceramidases).

Gangliosides

Gangliosides are a heterogeneous family of glycosphingolipids abundant in the brain. These glycolipids are composed of a glycosphingolipid (ceramide + oligosaccharide) with one or more sialic acids (n-acetyneuraminic acid) attached to the sugar chain. Over 40 gangliosides have been identified that differ mainly in the position and number of sialic acid residues. Gangliosides are important components of cellular membranes, and comprise ~ 6% of the total lipid content in brain. Gangliosides are differentially distributed in cells. For example, GD1a is more enriched in granule cells than in Purkinje cells, whereas the opposite is true for GT1a [17-19]. The ganglioside GD3 is heavily enriched in reactive and radial glia [17,20]. Glucosylceramide synthase (GCS; also known as glucosylceramide transferase) catalyzes the first glycosylation step in the biosynthesis of glycosphingolipids [21]. Human GCS was cloned in 1996 [22] and is primarily located to the golgi apparatus [21].

Evidence for Disturbance of Brain Lipid Content in Alzheimer's Disease

Phospholipids

It was first reported early in the 1990's that brain phospholipid content was decreased in AD. In particular, there were decreases in the phospholipid precursors choline, and ethanolamine with consequent decreases in levels of the associated phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in frontal and parietal cortex (brain regions severely damaged in AD), but not in the primary auditory cortex (a relatively unaffected brain region) [23]. Decreases in PC and PE were accompanied by increases in the phospholipid catabolite glycerophosphocholine, suggesting that decreases in these phospholipid species were due to increases in phospholipid turnover. Subsequent studies also found decreases in phosphatidylinositol that were specific to the fatty acids oleic and arachidonic in the hippocampus and parahippocampal gyrus of AD brains [24]. A number of these findings from autopsy brain have been supported by studies of cerebrospinal fluid (CSF) that used

this compartment as a window to study brain lipid metabolism in living patients. In CSF of AD patients, the choline metabolites glycerophosphocholine, phosphocholine and choline were decreased, as was the lysophosphatidylcholine/choline ratio compared with non-neurological disease controls [25,26], supporting data from brain tissues that there is an increased turnover of particular phospholipid species in AD. In addition to these region-specific losses of particular phospholipids, there may also be differences in brain phospholipid content that depend on the form of AD. Measures that determined total phospholipid content found decreases in frontal, temporal, caudate nucleus and hippocampus of patients with early onset AD, but no change in patients with late onset AD [27]. Although intriguing, it is difficult to determine if these differences are due to AD subtype or reflect the duration of AD, since there is evidence that oxidative stress and perturbed lipid metabolism peak early in the course of AD [28]. Moreover, measuring total phospholipid content could easily mask decreases in particular species of phospholipid. Thus, it is not clear at this time if there are differences in brain phospholipid metabolism that can be attributed to AD type. Together these results suggest that there is an increased metabolism of phospholipids in brain regions involved by AD, and that phospholipids containing choline and ethanolamine are most prominently involved. To date, few studies have addressed what particular phospholipid metabolic pathways are perturbed in AD, or the relationship of these pathways to the neuropathological correlates of AD.

Sphingomyelin, Ceramide, Sphingosine

While relatively few reports have directly examined sphingomyelin and ceramide levels in AD brain tissue and CSF samples, results thus far have consistently demonstrated that sphingomyelins are decreased and ceramides are increased in AD [29-32]. The most prominent changes appear to be in the very long-chain C24:0 and C24:1 species [31] that are enriched in endosomal lysosomal compartments, consistent with roles for ceramide in regulating β - and γ -secretases and APP processing in these compartments (sphingolipid regulation of APP processing is discussed in detail below). A recent report also demonstrated increased sphingosine with decreased sphingosine 1-phosphate (S1P) in AD brains compared with age-matched neurologically normal control subjects [30]. In general, higher S1P levels are protective to neurons [33,34] and elevations in sphingosine and ceramide can be toxic to neurons [31,35-38], suggesting that both increased levels of ceramide and a sphingosine/S1P rheostat imbalance contribute to neuronal dysfunction in AD. Indeed, expression of several genes involved in sphingomyelin and ceramide metabolism, including sphingosine 1-phosphate lyase, CerS1, 2 and 5, are increased in AD brain, as are genes involved in sphingomyelin catabolism including aSMase and factors associated with nSMase activation [39]. In addition, brain cells in AD patients exhibit decreased expression of genes involved in ceramide transformation that include acid ceramidase, ceramide kinase and genes involved in glycosphingolipid synthesis such as glucosylceramidetransferase [39]. However, only increased aSMase and nSMase have been confirmed at the level of enzyme activity and aSMase at the protein level [30]. In contrast to the findings from gene array, activity and protein levels of acid ceramidase were increased in AD brain [30]. While a great deal of work remains to positively identify sphingolipid metabolizing enzymes that are dysfunctional in AD, these initial reports suggest a pattern of enzyme dysfunction that favors ceramide generation at the expense of S1P and ganglioside synthesis. This altered sphingolipid profile is consistent with signaling that favors neuronal dysfunction and apoptosis.

Gangliosides

Reports of disturbances in glycolipids from AD brain first appeared in the late 1960s, when it was reported that particular fatty acid compositions of gangliosides, cerebroside and cerebroside sulfate levels were abnormal in AD brain [40,41]. Subsequent studies found

that the gangliosides GM1, GD1a, GD1b and GT1b were decreased in multiple brain regions including temporal white matter, temporal cortex and frontal cortex, whereas the gangliosides GM2, GM3, GQ1b and GT1L were increased in AD compared with age-matched control tissues [18,27,42,43]. Similar to the phospholipid findings discussed in the preceding section, disturbances in gangliosides are more profound in brain tissues from subjects with early onset disease, in which reductions of 58-70% were found in grey matter from nearly all brain regions, and an 81% reduction of total gangliosides in frontal white matter[44]. In late onset AD, gangliosides were selectively reduced in the temporal cortex, hippocampus, and frontal white matter [45]. Since GM1, GD1 and GT1 are enriched in neurons, reductions in these ganglioside species are thought to reflect synaptic and dendritic damage and frank neuronal loss in AD brain. Studies using CSF from living AD patients supports this hypothesis, with the observation that GM1 was increased in CSF of subjects with early-onset AD compared to late-onset AD and age-matched controls, likely due to a removal of GM1 from brain parenchyma [46]. Recent findings suggest that alterations in ganglioside metabolism may contribute to the development of neuropathologies associated with AD. For instance, GM1 is increased in detergent resistant membrane fractions isolated from frontal cortex of AD brain, and the gangliosides GM1 and GD1a have been found to co-label with dystrophic neurites, neurofibrillary tangles and A β -plaques [47-50]. Indeed, numerous studies in tissue culture and rodent models have shown that lipid composition is important for regulating pathways that promote APP cleavage and in regulating numerous aspects of synaptic function.

Membrane Lipids Regulate APP Processing

Specialized membrane domains known as lipid rafts are thought to play important roles in regulating the trafficking and proteolytic processing of the b-amyloid precursor protein (APP). These membrane microdomains contain mostly unsaturated lipids and are rich in sphingomyelin, gangliosides and cholesterol. This more “ordered” molecular arrangement results in a rigid structure that exhibits decreased lateral mobility compared with the surrounding phospholipid bilayer. Nevertheless, lipid rafts are dynamic structures that can coalesce to form larger platforms and separate into their component rafts within seconds to minutes [51,52]. There is evidence of considerable heterogeneity in the composition of lipid rafts, including variation in the lipid and sterol composition that is dependent on cell type, cellular location and activation state of the cell [51]. These dynamic membrane domains are involved in the regulation of protein trafficking and protein compartmentalization, and serve as organizational centers for the assembly of signaling complexes.

In AD, lipid rafts appear to be critical sites where the proteolytic processing of APP is regulated (Figure 2). Current evidence suggests that the non-amyloidogenic α -cleavage of APP occurs outside of lipid raft domains, where the disintegrin and metalloproteinase 10 (ADAM10; a major α -secretase in brain) is exclusively located [53]. Amyloidogenic processing of APP is thought to occur primarily in lipid rafts [54,55], where all relevant proteins appear to be concentrated including: BACE1, presenilins (PS1 and PS2), nicastrin, APH-1, PEN-2 (components of γ -secretase), APP, APP N- and N-terminal fragments and A β peptides [56-60]. These observations suggest that lipid rafts are major sites for amyloidogenic processing of APP. In this context, it is the trafficking of APP and secretases that regulate the form of APP processing. For instance, cellular glycosphingolipid levels are critical for regulating the maturation and cell surface transport of APP. Inhibition of glycosyl ceramide synthase (catalyzes the first step in glycosphingolipid biosynthesis) markedly reduced the secretion of APP and A β -peptide production, whereas the addition of exogenous gangliosides reversed these effects [61]. There is in addition, evidence that BACE1 and γ -secretase traffic in and out of lipid raft domains. While numerous studies found that BACE1 is located to membrane fractions consistent with lipid raft domains

[62-65], a closer examination has revealed that activation state plays a major role in regulating the membrane location of BACE1. Inactive BACE1 is located outside of lipid rafts, and active BACE1 is located to lipid rafts [66,67]. Forced targeting of BACE1 to lipid rafts by replacing the transmembrane and C-terminal domains with a glycosylphosphatidylinositol (GPI) anchor substantially upregulates the production of both sAPP β and A β , compared with cells over-expressing wild-type BACE1, consistent with a requirement for BACE1 to traffic into lipid rafts for β -cleavage of APP. Ceramide, a major bioactive component of lipid rafts, also appears to be important for regulating stabilizing BACE by facilitating the carrier-mediated translocation of acetyl-CoA into the ER lumen, where BACE1 is acetylated on seven lysine residues of the N-terminal portion of the protein. Site-directed mutagenesis experiments have shown that lysine acetylation is a necessary step for BACE1 to leave the ER and move ahead in the secretory pathway [68,69].

Similar to BACE1, γ -secretase is known to exist in both non-raft and lipid raft membrane domains and its activity is upregulated in lipid rafts. Increasing the sphingolipid and cholesterol content of proteoliposomes containing γ -secretase increased enzyme activity promoting the formation of lipid rafts [70]. In healthy cells and tissues γ -secretase is primarily located outside of lipid raft domains [62,66], where it is known to cleave the C-terminal fragments (CTFs) of Notch1, Jagged2, N-cadherin and deleted in colorectal cancer (DCC) [65]. Loss of γ -secretase activity by gene deletion or chemical inhibition results in the accumulation of APP CTFs in lipid rafts, indicating that γ -secretase cleavage occurs in these membrane microdomains [65]. With advancing age, and in AD, an increasing fraction of γ -secretase becomes located to lipid raft microdomains in post-Golgi and endosomal compartments [55,62,65,71]. Thus, the mislocalization of BACE1 and γ -secretase to lipid rafts may promote A β formation. Lastly, the membrane location of APP is important for determining the form of proteolytic processing, although little is known about the mechanisms that regulate the raft versus non-raft targeting of APP. One potential mechanism involves the interaction of APP with the cytoplasmic domain of low-density lipoprotein receptor-related protein (LRP), which is thought to promote BACE1-APP interaction by trafficking to lipid raft domains [72]. Thus, one potential explanation for the altered processing of APP to a pro-amyloidogenic pathway is that APP, β - and γ -secretases are increasingly localized to lipid rafts with increasing age and in neurodegenerative diseases [66]. However, there is also evidence that excluding BACE1 or γ -secretase from lipid rafts does not appreciably alter A β formation. In these experiments, it was found that BACE1 is S-palmitoylated at Cys⁴⁷⁴, Cys⁴⁷⁸, Cys⁴⁸², and Cys⁴⁸⁵, nicastrin is S-palmitoylated at Cys⁶⁸⁹, and APH-1 is S-palmitoylated at Cys¹⁸² and Cys²⁴⁵. Site-directed mutagenesis of these sites to Ala prevented S-palmitoylation and localized BACE1 and γ -secretase to non-raft membrane regions, but did not entirely disrupt the β - or γ -cleavage of APP or release of A β [73,74]. However, APP processing appears to occur less efficiently outside of rafts. Together, these data suggest that amyloidogenic processing of APP occurs most efficiently [62,66,75], but not exclusively [62] in lipid rafts.

Roles for Lipids in Seeding A β Aggregation

The self-aggregation of A β into oligomers and fibrils is a pivotal step in the pathogenesis of AD. There is considerable evidence that suggests particular lipids play key roles in regulating the conformational shift in A β from helix- to beta-sheet-rich structures (Figure 2). A β directly interacts with lipid membranes and disrupts the biophysical properties of the bilayer by disordering nearby lipids. Multinuclear NMR studies in model membranes have shown that the interaction of A β with lipid bilayers disrupts the lamellar phase of membranes and promotes a hexagonal phase with a significant number of non-oriented lipids [76]. The means by which A β interacts with lipid membranes involves a destabilization of A β structure, which leads to an extended peptide conformation that

increases the probability of electrostatic and hydrogen bonding interactions between the peptide and the head groups of lipids [77]. A β interacts with membranes in an electrostatic-independent manner [78], showing a preference for the head group of anionic lipids [79,80]. Using X-ray and neutron scattering techniques, it was found that A β exhibited enhanced interactions with charged lipids compared with zwitterionic lipids, and that these interactions promoted the formation of amyloid fibrils, suggesting that the interaction of A β with membranes seeded fibril formation [79]. Indeed, there is considerable evidence that lipid-interactions with A β are critical for fibril formation [80-85]. A recent study shed some light on how these complex interactions between lipids and A β are regulated. It was found that A β selectively bound to glycosphingolipids that contained a 2-OH group on the acyl chain of the ceramide backbone and did not effectively interact with glycosphingolipids that contained a nonhydroxylated fatty acid. Cholesterol inhibited the interaction of A β with glycosphingolipids that contained a 2-OH group, but increased the ability of A β to bind glycosphingolipids that contained nonhydroxylated fatty acids. Thus, cholesterol could either inhibit or facilitate interactions of A β with the membrane by regulating the form of glycosphingolipid that A β interacted with [86]. These studies are in agreement that the interaction of A β with membranes dramatically increases peptide aggregation rate, and induces a structural conversion in A β that favors peptide-peptide interactions to increase the probability of A β oligomerization from a random coil to the beta-structure observed in mature fibrils [80,81].

While A β interactions with anionic phospholipid head groups appear to be important for the interaction of A β with membranes, the interactions of A β with the ganglioside GM1 present in lipid rafts appears to have the greatest effect on A β aggregation and fibril formation. A β oligomers appeared rapidly after incubation with lipid rafts isolated from rodent brain, and this process was not disrupted by treatment with heat or proteinase K, suggesting that proteins contained in this lipid fraction were not critical for A β oligomerization [82]. However, increasing concentrations of the ganglioside GM1, either by direct addition or by incubating A β peptides with lipid raft fractions isolated from C2C12 cells rich in GM1 increases the rate of A β oligomerization, while incubation with ganglioside poor SK-N-MC cells slows the rate of A β oligomerization [82,83]. Interestingly, the removal of cholesterol from membranes does not eliminate the formation of A β oligomers [82]. Rather, the role for cholesterol in A β -oligomerization seems to be in the sterols' ability to promote the formation of GM1 clusters that preferentially interact with A β , compared with monomeric GM1, or other ganglioside species [84,85]. Recently, an intriguing study provided evidence that integrates A β interaction with phospholipid head groups (most abundant in less-ordered membrane regions) and GM1 (most abundant in ordered lipid raft regions). In this study it was found that exogenously applied A β can traffic on neuronal membranes and accumulate in lipid rafts by a mechanism dependent on the protein-tyrosine-kinase fyn [87]. Together these data suggest that A β binds to membranes with a preference for anionic lipid head groups, and A β is then incorporated into GM1-rich membrane regions where the peptides undergo a conformational shift that disrupts membrane stability and promotes peptide-peptide interaction and oligomer formation.

Roles for Deregulated Sphingolipid Metabolism in Disrupting Synaptic Activity

Synaptic dysfunction is an early and seminal event in the pathogenesis of AD. Disturbances of sphingolipid metabolism that occur in AD are likely to disrupt a number of protein-lipid interactions and perturb protein and lipid trafficking, and in so doing would dysregulate multiple cellular signaling events including those involved in synaptic plasticity/cognition (Figure 3). Sphingolipid metabolism is a dynamic process that modulates the formation of a number of bioactive metabolites including ceramide, ceramide-1-phosphate, sphingosine

and sphingosine 1-phosphate. It is becoming increasingly recognized that these sphingolipids play critical and complex roles in regulating neuronal excitability. The biophysical properties of sphingolipids play important roles in the regulation of membrane shape, endo- and exocytotic events, and vesicle and protein trafficking; several lipid species also function as second messengers that regulate neuronal functions including pre- and post-synaptic processes involved in synaptic plasticity [88-91].

At the presynaptic terminal, docking and fusion of synaptic vesicles with the plasma membrane is regulated by a highly conserved family of SNARE proteins that include synaptobrevin in vesicles and syntaxin and SNAP-25 at the plasma membrane. Recent findings have demonstrated that formation of the SNARE complex, synaptic vesicle fusion and exocytosis is regulated by sphingosine. Synaptobrevin adheres tightly to synaptic vesicles through electrostatic and hydrophobic interactions of the cytoplasmic part of synaptobrevin with vesicular membranes. Sphingosine activates synaptobrevin by neutralizing interactions of synaptobrevin with phospholipids, thus allowing synaptobrevin to engage syntaxin/SNAP-25, resulting in SNARE assembly, vesicle fusion and transmitter release [92]. These results are consistent with an earlier report that identified ceramidase (catalyzes the deacylation of ceramide to produce a free fatty acid and sphingosine) as an important regulator of synaptic vesicle exocytosis [93]. Sphingosine can be rapidly converted S1P by sphingosine kinase 1 (SK1), and this more soluble product has also been implicated in regulating transmitter release. SK1 is formed in the presynaptic terminal in an activity-dependent manner, and S1P applied to hippocampal neurons promotes transmitter release that is dependent on the S1P 1 receptor [94,95]. Roles for ceramide in neurotransmitter release that involve SMase activity have also been suggested in which the fusogenic properties of ceramide are thought to promote vesicle fusion with the plasma membrane [96-98].

Ceramide is emerging as an important regulator of synaptic function and has been implicated in synapse formation, transmitter release and plasticity [36,99-105] (Figure 3). Early evidence for the regulation of synaptic activity by ceramide was provided by experiments that used synthetic cell-permeable short (C2 – C6) ceramide analogs to demonstrate that ceramide could directly increase excitatory postsynaptic currents without affecting paired-pulse facilitation [104,106,107]. Interestingly, these ceramide-associated enhancements of excitatory currents were often transient and followed by sustained depression of excitatory postsynaptic currents [100,104,106,108,109]. One mechanism by which ceramide may be involved regulating synaptic activity is by controlling the spatial and temporal location of receptors at postsynaptic sites. In neurons, the sphingomyelin-, ceramide- and ganglioside-rich membrane regions known as lipid rafts have been identified as important sites for the docking and insertion of both NMDA and AMPA subtypes of glutamate receptors [52,99,110]. For instance, disrupting lipid rafts by removal of cholesterol from membranes inhibits NMDA receptor currents and calcium flux, and increases the basal internalization rate of AMPA receptors [111-113].

Although the precise mechanisms that regulate synaptic events by controlling lipid metabolism are still largely unknown, accumulating evidence suggests that the sphingomyelin catabolizing enzyme nSMase2 and its reaction product ceramide may play important roles. For instance, nerve growth factor (NGF) is known to regulate neurite outgrowth, synaptogenesis and to enhance the rate of depolarization-induced action potentials by signaling through nSMase2 and ceramide [36,105,114] [115-117]. In a similar fashion, TNF α can also enhance excitatory post-synaptic currents and NMDA-evoked calcium bursts by mechanisms that require nSMase2-regulated generation of ceramide and concurrent generation of diacylglycerol [99]. Ceramide's role in regulating synaptic activity may involve its fusogenic properties that would allow receptor insertion and removal. For

instance, ceramide may mediate focal changes in the biophysical properties of membranes that facilitate the traffic of transmembrane receptors [99]. Thus, a dysfunction of sphingolipids metabolism in AD could disrupt these sphingolipid-regulated pre- and post-synaptic events to perturb synaptic function.

Evidence for Ceramide-Assisted Neuronal Death in AD

There is considerable experimental evidence that cytokine dysregulation and increased cellular oxidation play key roles in neuronal death associated with AD [118,119]. There is also considerable evidence that pro-inflammatory cytokines, and cellular oxidants are important modulators of enzymes involved in sphingomyelin and ceramide metabolism. For instance, there is a bidirectional relationship between cytokine balance and ceramide levels. TNF α , IL-1, and Fas/FasL are potent inducers of ceramide production, and increased concentrations of ceramide can stimulate the production of IL-2 and IL-6 [120]. Oxidative stress is known to generate ceramide [38,121-124], and antioxidants such as N-acetylcysteine (NAC), pyrrolidine dithiocarbamate (PDTC), glutathione and α -tocopherol (vitamin E) have been shown to prevent the generation of ceramide induced by TNF α and Fas-ligand [122,125-128]. Sustained or excessive increases of ceramide can activate pro-apoptotic pathways. For example, Fas/FasL interaction can activate a caspase-8-dependent increase in SMase activity that increases ceramide that then promotes the formation of large lipid platforms and the assembly of cell death signaling protein complexes [129-132].

There are also several ceramide-regulated protein kinases (CAPK) and phosphatases (CAPP) that when activated can evoke signaling that triggers apoptosis. Pro-apoptotic CAPK signaling involves recruitment of MAPK/ERK kinase kinase (MEKK1), activation of SAPK-kinase (SEK1), Jun N-terminal kinases (JNK 1 and JNK2) and inhibition of the survival factor extracellular signal-regulated kinase-1 and 2 (ERK1 and ERK2) [131,133-137]. Signaling through the JNKs is thought to trigger apoptosis [136]. CAPK-induced apoptosis may also involve a Raf-1 kinase, mitogen-activated kinase (MEK-1) / ERK pathway that was first demonstrated in glia [138]. However, the contribution of Raf-1 signaling to neuronal death is unclear, and ERK can have protective or apoptotic effects depending on the mechanism of activation and duration of action. Potential roles for CAPPs in neuronal survival are not yet well defined, but may involve inactivation of the survival factor Akt1, p38 MAPK [139]. When taken together with the considerable evidence for the involvement of neuronal apoptosis in AD [119], the data described in this section support roles for perturbed membrane ceramide metabolism in this form of cell death.

Implications for Therapeutic Interventions that Target Shingolipid Metabolism

A working strategy for shingolipid metabolism-based interventions for the prevention and treatment of AD would be to develop drugs that suppress the excessive SMase-mediated cleavage of sphingomyelins that apparently occurs relatively early in the AD process. Preclinical proof-of-concept for the latter approach comes from studies showing that agents that inhibit sphingomyelin production (the serine palmitoyl-CoA-transferase inhibitor myriocin/ISP-1) [31] or an acid sphingomyelin hydrolysis (using D609) [140] can protect neurons against damage caused by Ab and oxidative stress. Another approach, more tailored towards disease prevention would be to prescribe diets that would be expected to lower levels sphingomyelin in brain cells. For example, diets rich in omega-3 fatty acids (fish, for example) would be expected to inhibit sphingomyelin metabolism and ceramide production [141], and may thereby protect neurons against synaptic dysfunction in AD as suggested from the results of preclinical trials in a mouse model of AD [142].

Summary

Our understanding of roles for sphingolipids in regulating neuronal function is increasing at a rapid rate, in part due to recent advances in our ability to measure and quantitate individual lipid species by mass spectrometry techniques and the availability of reagents to visualize and accurately modulate subcellular targets involved in sphingolipid metabolism. These advances have in addition led to a greater understanding of how perturbations in sphingolipid metabolism could contribute to the pathogenesis of neurodegenerative conditions such as Alzheimer's disease. Increased knowledge of the pathways that regulate sphingolipid metabolism and how these systems become dysregulated in Alzheimer's disease will ultimately lead to new therapeutics and disease prevention strategies that target these pathways.

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Figure 1.
Pathways of Sphingolipid Metabolism

Figure 2. Amyloid Processing in Lipid Rafts

Amyloid precursor protein (APP) cleavage by β - and γ -secretases occurs most efficiently in lipid rafts. $A\beta$ binds to membranes with a preference for anionic lipid head groups and can be translocated into GM1-rich lipid rafts where $A\beta$ undergoes a conformational shift that disrupts membrane stability, promotes peptide-peptide interactions and $A\beta$ oligomer formation.

Figure 3. Roles for Sphingolipids in the Regulation and Dysregulation of Synaptic Functions

Sphingosine can regulate neurotransmitter release by neutralizing interactions of synaptobrevin with phospholipids in synaptic vesicles. This action allows synaptobrevin to engage syntaxin/SNAP-25 on the inner leaflet of pre-synaptic membranes. A rapid and focal generation of ceramide by SMase promotes vesicle fusion with the plasma membrane and release of neurotransmitters. Sphingosine 1-phosphate (S1P) can signal in an autocrine manner through S1P receptors to further enhance the neurotransmitter release. At the post-synaptic terminal, rapid and transient increases of ceramide and DAG regulates plasma membrane trafficking of NMDA receptors by promoting the fusion of receptor-laden vesicles with the plasma membrane. In AD, disruptions of sphingolipid metabolism perturb these sphingolipid-regulated pre- and post-synaptic functions, and sustained increases of ceramide may activate ceramide associated protein kinases (CAPK) and protein phosphatases (CAPP) to promote death signaling in neurons.