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Review

Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction

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ABSTRACT

Simple sphingolipids such as ceramide and sphingomyelin (SM) as well as more complex glycosphingolipids play very important roles in cell function under physiological conditions and during disease development and progression. Sphingolipids are particularly abundant in the nervous system. Due to their amphiphilic nature they localize to cellular membranes and many of their roles in health and disease result from membrane reorganization and from lipid interaction with proteins within cellular membranes. In this review we discuss some of the functions of sphingolipids in processes that entail cellular membranes and their role in neurodegenerative diseases, with an emphasis on SM, ceramide and gangliosides.

1. Introduction

Ceramide constitutes the backbone of more complex sphingolipids (Fig. 1) and is at the hub of sphingolipid metabolism (Fig. 2). The attachment of phosphocholine or saccharides to the hydroxyl group of ceramide in the Golgi apparatus leads to the production of sphingomyelin (SM) and glycosphingolipids (GSLs), respectively. Gangliosides are GSLs that contain sialic acid [1,2].

The nervous system of mammals is rich in SM [3] and GSLs [2]. Gangliosides are major components of neuronal membranes, where they contribute up to 10–12% of the total lipid content and participate in crucial processes of the nervous system [2]. Their regulatory roles are suggested by the dramatic change in the pattern of gangliosides expressed during development of the nervous system, as well as by the region-specific distribution of gangliosides with different carbon chain length in the ceramide moiety [4]. The brain content of SM, ceramide and GSLs changes throughout life, during aging and in neurodegenerative diseases; some of these changes are discussed below.

2. Sphingolipids: master regulators of membrane dynamics

Sphingolipids have a natural predisposition to lateral segregation within membranes and to the formation of membrane microdomains (or lipid rafts) that are also enriched with cholesterol. The unique conformation of the amide group and the hydroxyl group on C3 of the ceramide moiety allows for the establishment of a network of hydrogen bonds at the water/lipid interface that stabilizes sphingolipid-enriched microdomains. Lateral segregation of gangliosides and other GSLs is further favored by the formation

Abbreviations: APP, amyloid precursor protein; BACE, β-site APP-cleavage enzyme; caspr, contactin-associated protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; GSLs, glycosphingolipids; IL1β, interleukin 1β; JNK, c-Jun-N-terminal kinase; NCX, sodium–calcium exchanger; Neu3, N-acetyl-α-neuraminidase 3; NF-155, neurofascin-155; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NT-3, neurotrophin 3; PAG-N, phosphoprotein associated with glycospingolipid-enriched microdomains; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; ROS, reactive oxygen species; SBD, sphingolipid-binding domain; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SFK, Src-family kinase; SM, sphingomyelin; aSMase, acidic sphingomyelinase; nSMase, neutral sphingomyelinase; TAG-1, transient-axonal glycoprotein 1; mTOR, mammalian target of rapamycin; Trk, tropomyosin-receptor kinase; TRPC5, transient receptor potential cation channel subfamily C member 5

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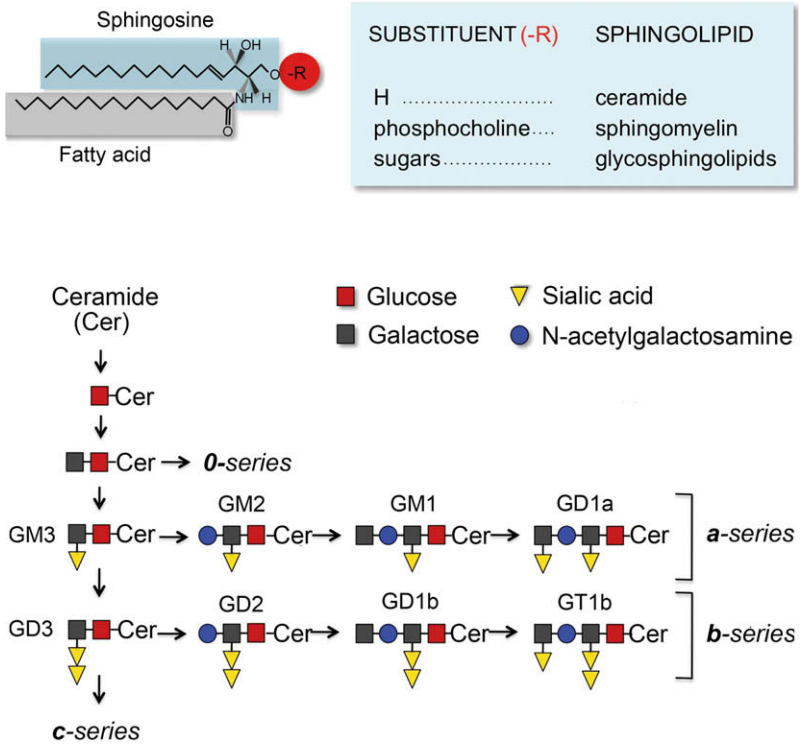

Fig. 1. Structure of sphingolipids and ganglioside biosynthesis pathway. Sphingolipids derive from long-chain amino alcohols (sphingoid bases). Complex sphingolipids and gangliosides share a ceramide backbone formed by the sphingoid base (mainly sphingosine) linked to a long-chain fatty acid (C:16, C:18 or longer). The primary alcoholic group of ceramide is attached to phosphocholine or saccharides, producing sphingomyelin, and glycosphingolipids, respectively. One or more saccharides may be linked to ceramide. Gangliosides are mono- or multi-sialosylated glycosphingolipids. Their synthesis is a multi-step process in which a sugar is first added to ceramide, followed by stepwise addition of other saccharide residues to the growing glycan. The major gangliosides in the brain are GM1, GD1a, GD1b and GT1b.

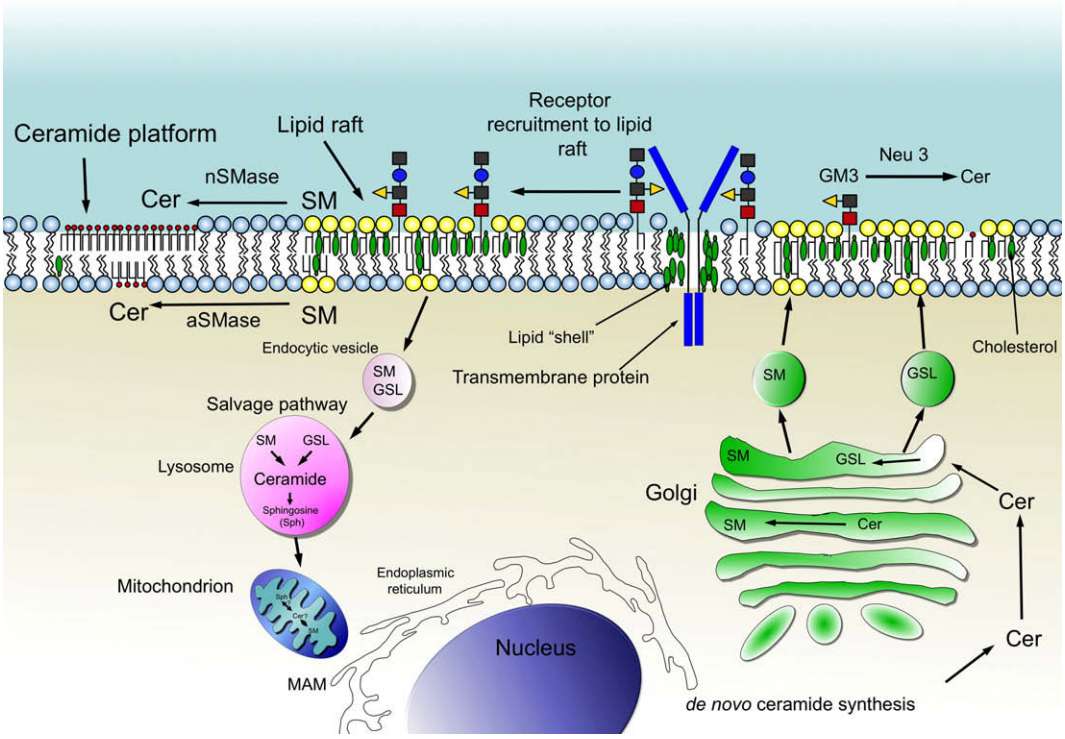

Fig. 2. Compartmentalization of sphingolipid and ganglioside metabolism. Ceramide synthesized in the endoplasmic reticulum is transported to the Golgi apparatus where it is converted to SM and GSLs. From the Golgi, SM and GSLs are transported by vesicular transport to lipid raft domains at the plasma membrane. SM is converted to ceramide by acid or neutral SMases. Ceramide segregates laterally forming big ceramide platforms poor in cholesterol. GM3 is also converted to ceramide by the action of sialidases and glycohydrolases. Also represented is the interaction of transmembrane proteins with gangliosides in the formation of lipid shells that favors the recruitment of proteins to lipid rafts. SM and GSLs catabolism at the lysosomes provides ceramide and sphingosine to the 'salvage' pathway. In particular circumstances, ceramide can also be derived from sphingomyelin in the mitochondria.

of hydrogen bonds between adjacent sugar residues. Lipid rafts play very important roles in a variety of cellular processes including membrane trafficking, signal transduction, cytoskeletal organization, etc. The biophysical and physiological aspects of lipid rafts are discussed elsewhere in this special issue.

Proteins that associate with lipid rafts tend to have glycosyl phosphatidylinositol (GPI) anchors, or to be dually acylated, palmitoylated, or associated to cholesterol. Transmembrane proteins may have cholesterol and sphingolipid-containing "lipid shells" that stably interact with specific aminoacid residues in the transmembrane domains of the protein, and that favor protein segregation into lipid rafts (Fig. 2). These protein-specific lipid shells, are stabilized by the specific interaction of the sugar headgroup of the ganglioside with the protein oligosaccharide chains or aminoacids [5]. Because 'raft' proteins tend to have reduced lateral mobility, protein–protein interactions are facilitated within particular lipid microdomains.

The roles of sphingolipids in biological membranes, however, extend well beyond the formation of lipid rafts. In fact, sphingolipids are highly dynamic lipids that modulate membrane composition and behavior through their reciprocal interconversion and the formation of specific lipid–protein interactions. For instance, local conversion of SM to ceramide, by the action of one of several SMases[1] at the plasma membrane, is a regulated event that has profound effects on the biophysical properties of the membrane and on cell function. Many cellular functions of ceramide have been explained based on these changes [6]. Since ceramide mixes more poorly than SM with glycerophospholipids and cholesterol [6,7], SM conversion to ceramide results in the formation of large ceramide-enriched domains (ceramide platforms) that exclude cholesterol and alter the composition of lipid rafts (Fig. 2) [7–10].

Ceramide-induced lateral lipid separation has also profound effects on membrane proteins. Different proteins are recruited or excluded from ceramide platforms depending on their relative affinity for cholesterol and ceramide. One consequence of this differential affinity is the clustering of plasma membrane receptors upon ceramide generation, a phenomenon studied in detail for receptors such as Fas and CD40 [6]. A second consequence of the changes in membrane microdomains that results from SM conversion to ceramide is the specific recruitment of ancillary proteins to receptor complexes. That is the case for the recruitment of caveolin-1 to phosphoinositide 3-kinase (PI3K)-associated receptor complexes within lipid raft, which causes inactivation of PI3K [11].

Ceramide generated from SM at the plasma membrane also induces pore formation and increases membrane permeability through transmembrane ceramide channels, causing abnormal flux of calcium and other ions that affects the activity of local enzymes [6,12]. In addition, the change of membrane curvature that takes place upon ceramide accumulation could also alter the three-dimensional structure of membrane enzymes causing their activation or inhibition [6]. Finally, lateral lipid separation and ceramide-rich domain formation create membrane-packing defects that might allow and/or facilitate the interaction of ceramide with its direct target proteins.

Contrary to the well-defined regulatory roles of the SM cycle, the conversion of gangliosides to ceramide at the plasma membrane has only started to be appreciated lately. Ceramide can be generated at the plasma membrane from ganglioside GM3 by the combined action of the sialidase N-acetyl-α-neuraminidase 3 (Neu3), which is highly expressed in the brain, and membrane-bound glycosylhydrolases [13]. In principle, ganglioside-derived ceramide would trigger the same changes in membrane dynamics that are induced by SM-derived ceramide, although the size of the ceramide pool generated from gangliosides could be smaller. However, the cellular outcome would be very different due to the concomitant decrease of ganglioside levels and the consequent loss of specific lipid–protein interactions. The sialidase Neu3 is also responsible for the modulation of plasma membrane ganglioside composition in a focal and regulated manner. As discussed later, these changes may profoundly affect cell signaling.

3. Modulation of membrane protein activity and signaling

Sphingolipids and gangliosides are important modulators of membrane receptors, ion channels and downstream signaling pathways. Regulation occurs by different mechanisms, some of which are rather general and others that are receptor and/or ganglioside-specific. The list of membrane proteins regulated by sphingolipids and gangliosides will likely grow as we increase our understanding of membrane dynamics and protein–lipid interactions.

3.1. Regulation through changes in the membrane composition

A general mechanism of regulation of receptors and ion channels by sphingolipids and gangliosides involves their recruitment or exclusion, along with associated proteins, to specific GSL- or ceramide-enriched membrane microdomains. The composition of these domains affects protein–protein interactions and/or receptor trafficking. This is probably the case for the platelet-derived growth factor (PDGF) receptor, the activation of which is inhibited by most complex gangliosides. Experimental overexpression of GM1 blocks PDGF receptor signaling by displacing it outside of lipid rafts [14], suggesting that local accumulation of GM1 might modulate receptor function. In physiological conditions, focal increase of GM1 might result from regulated activation of Neu3 by the transmembrane adaptor protein phosphoprotein associated with glycospingolipid-enriched microdomains (PAG-N) in caveolin-associated microdomains [15]. Thus, although the mechanism is somehow general (movement of the receptor outside of lipid rafts) the trigger of ganglioside accumulation is specific.

A common mechanism of membrane protein regulation by ceramide involves the formation of ceramide-rich platforms upon activation of SMases. An example is the activation of tropomyosin-receptor kinase A (TrkA) by ceramide in the absence of the TrkA ligand nerve growth factor (NGF) (reviewed in [16]). This activation takes place within low-buoyancy membranes, enriched in ceramide and with low cholesterol content.[2] In this particular case the recruitment of TrkA to ceramide-rich domains would be sufficient for receptor activation based on the evidence that TrkA dimerizes and autoactivates in the absence of NGF when its concentration at the plasma membrane reaches a high enough level [17].

The mechanism of membrane protein regulation by formation of ceramide-rich platforms has been studied in detail in the case of ceramide-induced Kv1.3 inhibition. Kv1.3 belongs to the group of shaker channels, which in the central nervous system are found primarily in axons where they modulate electrical activity. Kv1.3 channels require raft localization for proper function. The formation of ceramide-rich platforms inhibits Kv1.3 activity directly by changing the lipid composition of rafts, and possibly indirectly by bringing together the channel with Src-like tyrosine kinases (SFK), which cause phosphorylation of Kv1.3 [18]. Several other ion channels are regulated by ceramide in a similar lipid raft/SFK dependent fashion [19]. The "recruitment" effect to ceramide platforms is not specific to SFK, and could also explain the activation of other signaling

[1] For the purpose of this review we will not differentiate in detail the different SMases.

[2] Posse de Chaves et al., unpublished observations.

intermediates involved in ceramide-mediated regulation of ion channels, such as c-Jun-N-terminal kinase (JNK) [20] and protein kinase C (PKC) [21]. For example, the rapid and focal generation of ceramide downstream of the inflammatory cytokine TNFα determines activation of the N-methyl-D-aspartate (NMDA) receptor and excitatory post-synaptic currents by recruiting in close proximity and clustering into ceramide-rich platforms both the NMDA receptor subunit NR1 [22] and the kinases that phosphorylate it, PKC and PKA.

The inflammatory cytokine interleukin (IL) 1β regulates ion channels activity in a similar manner. Binding of interleukin 1β (IL1β) to its receptor IL1R1 activates SMases and ceramide production [23]. Ceramide mediates the fast, non-transcription-dependent effects of IL-1β [24,25], which have been linked to the development of epilepsy [26], seizures [27] and rapid induction of fever [24]. Recruitment of Src kinases to the IL-1 signaling complex leads to ion channels phosphorylation pre- or post-synaptically [25]. In particular, tyrosine phosphorylation of NMDA receptor NR2B subunit mediated by ceramide-activated SFK is responsible for the pro-convulsive effects of IL-1β [27].

Like ceramide, GSLs modulate the activity of SFKs through recruitment into lipid rafts. Gangliosides (GD1b in particular) are closely associated to c-Src and Lyn in lipid rafts [28]. The outcome of the association of these signaling molecules with GSL-enriched microdomains, may still depend on cell context and the contemporaneous recruitment of activating or inhibiting factors in the same microdomains. How GSLs located in the outer leaflet of the plasma membrane interact with proteins in the inner leaflet is still unclear, but recent evidence suggest that the length of the fatty acid moiety linked to the GSL may be the key element, with GSLs containing long C24:0 fatty acids being able to interdigitate with raft-anchored SFKs (reviewed in [29]).

3.2. Lipid–protein interactions

A second modality of regulation of membrane receptors by sphingolipids and gangliosides entails specific interactions of lipids with the receptors. One of the best-characterized models is the modulation of epidermal growth factor receptor (EGFR) activity by the ganglioside GM3. GM3 inhibits EGFR signaling without interfering with EGF binding or receptor dimerization. This effect requires the sugar headgroup of GM3, which interacts with an oligosaccharide chain in the extracellular domain of EGFR [30]. In this case GM3 works as a protein-specific escort. Rather than directly inhibiting receptor activation, GM3 might favor receptor inactivation by targeting GM3-bound EGFR to specific membrane domains that contain a Receptor Tyrosine Phosphatase (RTPase) responsible for receptor dephosphorylation (reviewed in [31]). Insulin receptor (IR) is another tyrosine kinase receptor that is inhibited by the ganglioside GM3. Inhibition is mediated, at least in part, by the specific interaction of GM3 with a critical lysine residue in the receptor [32]. This interaction disrupts the binding of IR to caveolin, which is crucial for IR signaling, and leads to insulin resistance. In line with this observation, mice lacking GM3 have increased insulin sensitivity [33].

Gangliosides, in particular GM1, potentiate the action of neurotrophins and may activate Trk receptors even in the absence of neurotrophins [34]. Like in the case of EGFR, glycosilation of TrkA receptor seems to play a role in the interaction with GM1 [35]. GM1 might facilitate dimerization and receptor phosphorylation in a manner similar to ceramide accumulation. Receptor/ganglioside interaction could also have a role in the trafficking and targeting of TrkA receptor to the plasma membrane [36].

While the interactions described above are mediated by glycans and oligosaccharide chains, some proteins contain specific sphingolipid-binding domains (SBD) with highest affinity for galactosylceramide and SM. Examples include the nicotinic acetylcholine receptor, PDGF receptor, Thy-1, the HIV-1 surface envelope glycoprotein gp120 and the Aβ peptide. In the case of the nicotinic acetylcholine receptor, the specific interaction between SBD and sphingolipids result in conformational changes important for receptor assembly, trafficking and function [37].

3.3. Gangliosides as co-receptors

A third regulatory mechanism peculiar to gangliosides involves their function as co-receptors that bind ligands and expose them to the main receptor in a proper, specific orientation. An example is given by the effect of GM1 on the fibroblast growth factor 2 (FGF2) receptor. GM1 has a bimodal action on this receptor: in cells that do not synthesize heparan sulfate proteoglycans (necessary for ligand presentation and binding), administration of GM1 activates FGFR by directly binding FGF and allowing ligand binding to FGFR. However, in normal cells, administration of GM1 has a general negative effect on FGFR signaling, perhaps due to the competition between heparan sulfate and GM1, or to changes in membrane composition that result from GM1 enrichment. A co-receptor role for gangliosides has been proposed also in the case of serotonin and possibly other neurotransmitters with amino groups (GABA, glutamate and some peptides such as enkephalin and substance P). Binding of serotonin to GM1 stabilizes neurotransmitter monomers and might orient them close to the membrane for optimal binding to serotonin receptors (reviewed in [37]).

4. Cell adhesion/recognition and myelin–axon interactions

GSLs are involved in carbohydrate–carbohydrate and carbohydrate–lectin interactions that are key to the formation of "glycosynapses" and essential to cell–cell recognition, adhesion and signaling. As originally defined by Hakomori, glycosynapses are membrane domains that are distinct from classical lipid rafts since they may exist independently of cholesterol. In the glycosynapses, the glycan structure of the gangliosides and the glycosylation state of tetraspanins are essential for the interactions that bring together and bridge specific extracellular proteins, tetraspanins, integrins and other adhesion proteins, and signaling molecules. For instance, interaction of GM3 with the tetraspanin CD9 leads to the recruitment of integrin α3 in the complex and to inhibition of cell mobility. Furthermore, high levels of GM3 also recruit the Src kinase csk into the glycosynapse, resulting in phosphorylation and inhibition of c-Src [38].

Even outside classical glycosynapses, gangliosides facilitate the formation of supramolecular complexes where multiple signals are integrated. The result is often a potentiated signal. For example, two important converging signals for neurite outgrowth in many neural cells are activation of TrkA receptor by NGF and interaction of the extracellular matrix protein laminin-1 with integrins. TrkA activation alone is not enough to produce rapid and robust neurite outgrowth. On the other side, induction of neurite outgrowth by laminin requires the presence of NGF. Cooperation between the two pathways is achieved by binding of laminin-1 to GM1, induction of ganglioside clustering and relocation of TrkA and β1 integrin to lipid rafts. Here, the subsequent recruitment of downstream signaling molecules such as Lyn, Akt and MAPK leads to neurite outgrowth [39]. This peculiar role of GM1 might explain why during axonal development, the concentration of GM1 is focally increased by sialidase Neu3 at the tip of a single neurite, leading to activation of TrkA and axonal growth [40].

One aspect of ganglioside-mediated cell–cell interaction with paramount importance in the nervous system is the organization of myelinated nerve fibers. The interaction between myelin sheet

and axon is critical not only for the fast saltatory conduction of action potentials, but also for axonal trophic support. Myelin sheets can still form normally in the absence of ganglioside synthesis in oligodendrocytes [41]. However, neuronal gangliosides, and in particular GM1, GD1a and GT1b, are essential for the maintenance of myelinated axons in the central and peripheral nervous system. GM1, which is enriched in paranodal structures (Fig. 3), is critical for lipid raft partitioning and compartmentalization of the glial neurofascin-155 (NF-155) and the axonal contactin/caspr1 (contactin-associated protein 1) proteins. These are two adhesion proteins that interact with each other in *trans* and that are essential for the cytoarchitecture of paranodal regions (reviewed in [42]). In GM2/GD2 synthase null mice, lack of GM1, among other complex gangliosides, results in reduced expression of caspr and NF-155 and disruption of paranodal junctions as it occurs in Caspr and NF-155 knock-out mice [43].

In myelinated fibers GM1 could potentially modulate the activity of a third adhesion molecule, transient-axonal glycoprotein 1 (TAG-1), which is highly expressed in the juxtaparanodes and is essential for correct localization and compartmentalization of the Kv1.1/1.2 (Shaker-like) channels (reviewed in [42]). TAG-1 and GM1 do associate in cerebellar granule cells [44] and TAG-1 activity depends on gangliosides in the cerebellum [45]. However, the interaction between TAG-1 and GM1 has not been investigated in myelinated fibers, yet.

Interestingly, reduced expression of NF-155 and disorganization of paranodal structures have been observed in early lesions in the brain of patients with multiple sclerosis (MS) prior to demyelination. These abnormalities are accompanied by decreased expression of GSLs, galactosylceramide, sufatides and GM1 in particular, reorganization of lipid rafts and redistribution of NF-155 outside of lipid rafts [46]. The underlying mechanisms are not clear and the question remains whether at least some of these abnormalities might be caused by the presence of antibodies against GM1 and other gangliosides in the serum of MS patients, or by increased ganglioside-specific T and B cell reactivity [47,48].

Myelin stability and proper axonal function also depend on the interaction of GD1a and GT1b, the two most abundant gangliosides in axonal membranes, with the myelin-associated glycoprotein (MAG), a sialic acid-binding protein belonging to the family of Siglecs. GD1a and GT1b are specific MAG ligands and their absence in the myelinated fibers of GM2/GD2 synthase null mice results in impaired phosphorylation of axonal neurofilaments, aberrant neurofilament spacing and decreased axon caliber [49]. Not surprisingly, GM2/GD2 synthase null mice develop a neurodegenerative phenotype that begins with axonal degeneration and demyelination in the central nervous system and in the sciatic nerve [50]. Recently, the specific binding of MAG to GD1a and GT1b has been shown to confer protection against axonal toxicity induced by vincrestin and other neurotoxins, perhaps through microtubule stabilization in the axons [51].

As axonal receptors for MAG, the gangliosides GD1a and GT1b have been implicated in the MAG-mediated inhibition of axonal outgrowth and regeneration following axonal injuries or disease. However, MAG interacts with other receptors on the axolemma, including Nogo receptor (NgR), and can inhibit axonal growth by engagement of these receptors in a ganglioside-dependent or independent manner, based on cell context. This topic is reviewed elsewhere in this special issue.

5. Sphingolipids in intracellular membranes and organelles

In addition to their crucial roles at the plasma membrane, sphingolipids can also modulate the structure and function of intracellular membranes and processes that involve intracellular organelles.

5.1. Endosomes and endocytosis

Different mechanisms of endocytosis have specific protein requirements such that they depend on dynamin, clathrin, and/or caveolin. The requirements for lipids are also different, i.e. general

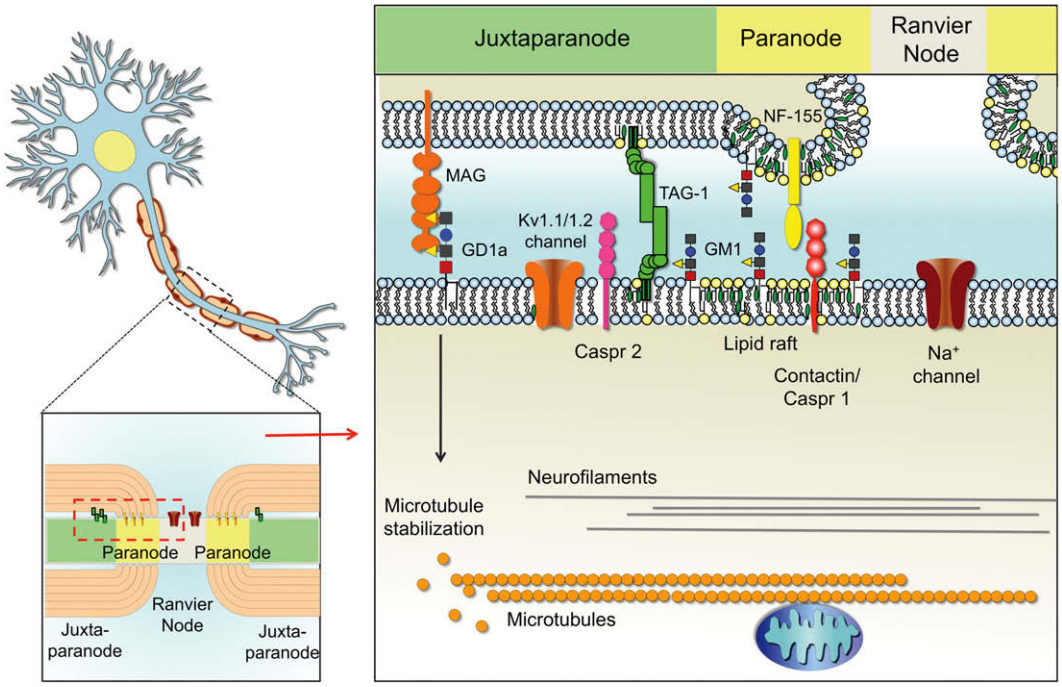

Fig. 3. Role of gangliosides in myelin–axon interactions. The molecular and cytoarchitectural structure of paranodes is key to the spatial separation of K^+ and Na^+ channels and to nerve conduction. GM1 is enriched in lipid rafts at the paranodes, where it mediates compartmentalization of the adhesion proteins NF-155 and Contactin/Caspr1. Interaction of MAG with GD1a stabilizes myelin–axon interaction and might also have a neurotrophic function on axons, perhaps through microtubule stabilization.

depletion of sphingolipids inhibits multiple mechanisms of clathrin-independent endocytosis but not clathrin-dependent uptake, and selective depletion of GSLs inhibits only caveolar endocytosis [52]. Conversely, acute treatment with exogenous natural or synthetic GSLs stimulates caveolar endocytosis without affecting other endocytic mechanism [53]. The mechanism(s) by which GSLs enhance caveolar endocytosis are not well understood, but Pagano's group has proposed two models [53]. The first model suggests that exogenously added GSLs may interact as membrane environment modifiers or as ligands for specific plasma membrane proteins, which, in turn, would initiate a cascade of signaling events resulting in activation of caveolar endocytosis. The second model predicts that addition of GSLs at the outer leaflet of the plasma membrane would change the lipid distribution and organization at both leaflets of the plasma membrane, which in turn could induce a redistribution of lipid-modified signaling proteins on the inner leaflet of the plasma membrane. These changes would cause the activation of kinases at the plasma membrane and initiate caveolar endocytosis.

As for many ceramide functions, the role of ceramide in the regulation of endocytosis is cell-type specific. The intrinsic ability of ceramide to promote the lamellar to non-lamellar phase transition promotes vesicle budding, membrane fusion, and ATP-independent endocytosis [6,7]. Indeed, it has been suggested that cells may use SMase activation as a mechanism to regulate the local lipid composition and to promote endocytosis [54]. In line with this hypothesis, neurons isolated from mice lacking acidic sphingomyelinase (aSMase) have increased content of plasma membrane SM in lipid rafts and show aberrant localization of GPI-anchored proteins and GM1 to dendrites due to defective membrane internalization in these structures [55].

An increase of membrane fusion/fission events due to ceramide elevation at the plasma membrane could explain some subcellular features present in the endosomal/lysosomal system during normal aging or in certain neurological disorders such as Alzheimer's disease (see Section 6). In contrast to the notion that ceramide enhances endocytosis, we found that ceramide inhibits uptake of NGF [56]. The inhibitory effect of ceramide could be explained by a miss-localization of NGF receptors at the plasma membrane. Indeed, NGF-activated TrkA but not ceramide-activated TrkA moves out of rafts to allow its internalization through clathrin-mediated endocytosis[3] due to changes of membrane microdomains that result from ceramide accumulation.

5.2. Mitochondria and apoptosis

The nervous system has high-energy demands, which at the neuronal cellular level become even more pronounced due to the extremely polarized nature of neurons and the specific needs in precise cellular compartments such as synapses. Impairment of mitochondria function has dramatic effects on neural tissue, and mitochondria are indeed greatly affected in many neurodegenerative disorders. In addition, mitochondrial membranes act as 'sensors' of cellular stress and respond with changes that culminate in apoptosis. Mitochondria are also the main targets and mediators of the pro-apoptotic role of ceramide and ganglioside GD3 in certain conditions.

While gangliosides are not normally detectable in brain mitochondria, a pool of mitochondrial ceramide is physiologically present, and more abundant in synaptosomal than in non-synaptosomal mitochondria [57]. The pool of mitochondrial ceramide might have multiple origins: synthesis in situ from sphingosine, cleavage of mitochondrial SM, or direct exchange from ER or mitochondria associated membranes (MAMs) (Fig. 2) [58,59]. Importantly, SM present at the mitochondrion, and not at the outer leaflet of the plasma membrane, seems to provide the pool of ceramide involved in apoptosis [58]. Ceramide influences several aspects of mitochondria function. It increases mitochondrial and cytosolic Ca++ concentrations, increases the formation of reactive oxygen species (ROS), causes ATP depletion, induces a collapse of mitochondrial membrane potential ($\Delta\Psi m$), and induces the release of mitochondrial proteins to the cytosol [60,61]. Some of the mechanisms by which ceramide alters mitochondrial activity directly involve mitochondrial membranes, such as the formation of pores that serve as channels for large proteins. Other mitochondria-related processes affected by ceramide are due to the second messenger role of ceramide, in particular inhibition of PI3K/Akt pathway, activation of protein phosphatase 2A (PP2A) and activation of JNK [16,58,61]. Once again, which mechanism prevails in the mitochondrial effects of ceramide is dependent on the cell type.

GD3 ganglioside is an apoptotic mediator in numerous cell types including neurons and glial cells. GD3 is actively synthesized and accumulates early and transiently during apoptosis. GD3 targets mainly mitochondria causing significant mitochondrial function changes, similar to those induced by ceramide. GD3 increases production of ROS, dissipates the mitochondrial membrane potential ($\Delta\Psi m$), and induces release of apoptogenic factors such as cytochrome c, ATP, AIF, and caspases. In addition, GD3 produced upon exposure to Aβ seems to be responsible for re-activation of the cell cycle in neurons [62], a condition that precedes neural cell death. GD3 accumulation requires ceramide produced by the action of acidic SMase, which in turns promotes the synthesis and relocalization of GD3 to mitochondria [63]. At the mitochondria, GD3 would induce changes in membrane viscosity and curvature, which could result in mitochondrial fission. It has been hypothesized that the small fragments of mitochondria could act as "cargo boats" transporting GD3 and other pro-apoptotic molecules into the nucleus [64]. A recent report suggests the possible consequences of GD3 synthase regulation in human disease by demonstrating that elimination of GD3 synthase improves memory and reduces Aβ plaque load in APP/PSEN1 mouse model of Alzheimer's disease, and that in primary neurons and astrocytes lacking GD3 synthase Aβ-induced cell death is inhibited [65].

5.3. Autophagy

Autophagy represents a very important cellular process that involves extensive intracellular membrane rearrangement in which membrane dynamics play a pivotal role. Although originally seen as a cellular resource activated under conditions of nutrient starvation, autophagy also provides an essential mechanism for the clearance of abnormal and misfolded proteins. There is now strong evidence that autophagy may represent a key neuroprotective mechanism in many neurodegenerative conditions, including AD, PD and Huntington's disease. Ceramide and gangliosides regulate autophagy in a positive manner. The mechanisms involved in the induction of autophagy by ceramide and gangliosides include regulation of protein expression, i.e. upregulation of the autophagy gene beclin 1 [66,67] or downregulation of nutrient transporter proteins [68], and inhibition of signaling molecules such as protein kinase B [67]. None of these mechanisms seems to be linked directly to ceramide effects on membrane dynamics. However, mechanisms involving ceramide induction of membrane curvature have also been proposed [69].

Autophagy is enhanced in the brain of mice models of GM1-gangliosidosis [66], while pharmacological reduction of gangliosides levels in a cellular model of synucleinopathy causes autophagy inhibition, due to impaired fusion between autophagosomes and lysosomes, downregulation of Beclin-1 and ATG5, and

[3] Posse de Chaves et al., unpublished observations.

upregulation of the negative regulator of autophagy mammalian target of rapamycin (mTOR) [70]. The implications of autophagy induction for cell fate are complex. Autophagy is indeed a Janus-faced process which has protective roles in aging, neurodegenerative diseases and infectious diseases, but may also represent a true mode of cell death under specific circumstances [71].

5.4. Endoplasmic reticulum-nuclear membranes and Ca^{2+} homeostasis

The transduction of extracellular stimuli to intracellular responses by mechanisms that involve Ca^{2+} signaling is of vital importance in a wide range of cellular processes. Given the function of sphingolipids in cell signaling it is not surprising that they also contribute to regulation of cellular Ca^{2+} homeostasis. In most cases their role is indirect, through modulation of signaling pathways that lead to elevation of intracellular $[Ca^{2+}]_i$ in a cell-specific manner. However, gangliosides may also have a direct effect, by interacting with and modulating the properties of Ca^{2+} channels (reviewed in [72]). In this regard, work by R. Leeden and collaborators have demonstrated that GM1 (but not other gangliosides) binds to the sodium–calcium exchanger (NCX) in the nuclear membrane, and regulates calcium transfer from nucleoplasm to endoplasmic reticulum [73]. This function of GM1 is important to maintain calcium homeostasis, as demonstrated by the fact that lack of GM1 and impaired regulation of NCX activity result in increased susceptibility of GM2/GD2 synthase null mice to kainate-induced seizures, excitotoxicity and apoptosis [72]. The recent discovery that the sialidase Neu3 is also present in the inner nuclear membrane, along with gangliosides GM1 and GD1a, suggests that this enzyme may play a regulatory function by raising the local concentration of GM1 and stimulating NCX activity [74].

Gangliosides might also modulate the activity of plasma membrane and endoplasmic reticulum Ca^{2+}-ATPase. Both GM3 and GM1 have been shown to affect protein conformation and activity of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in rabbit skeletal muscle, although in opposite directions, with GM3 playing a stimulatory and GM1 an inhibitory effect [75]. The significance of these findings in the context of normal cell physiology is not clear, since these studies were performed by adding exogenous gangliosides to membrane preparations, thus increasing ganglioside membrane content well beyond physiological concentrations. However, they may help explaining why in lysosomal storage disorders such as Sandhoff disease, in which cells fail to degrade GM2, accumulation of gangliosides in the ER causes altered SERCA pump function and impaired calcium homeostasis [76].

GM1 is also involved in modulating the activity of voltage-independent Ca^{2+} channel transient receptor potential cation channel subfamily C member 5 (TRPC5) at the plasma membrane, although with an indirect mechanism. Experimental cross-linking of GM1 in neuroblastoma cells results in co-clustering of $\alpha5\beta1$ integrin in a complex with GM1, activation of PLCγ and PI3K, and calcium influx through the TRPC5 channels [77].

The effect of ceramide on the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) varies depending on the cell type and it is usually indirect.

6. Lessons from diseases and models of sphingolipid depletion

In spite of the overwhelming evidence of the role of sphingolipids in membrane dynamics and cell signaling, there are surprisingly few recognized pathologies due to the impairment of sphingolipid biosynthesis. No inherited deficiency of ceramide synthase has been reported so far. GM2/GD2 synthase deficiency (leading to lack of complex gangliosides and accumulation of the precursor GM3) has been described in a single patient with impaired motor development, seizures and death in infancy [78]. Loss-of-function mutation in the gene encoding GM3 synthase causes another extremely rare autosomal recessive disorder, the infantile-onset symptomatic epilepsy syndrome, which is characterized by progressive brain atrophy, epilepsy, chorea [79]. The corresponding murine knock-out models lacking GM2/GD2 synthase (lack of complex gangliosides of the 0-, a- and b-series) or GM3 synthase (lack of a- and b-series gangliosides), display a different phenotype, with motor and behavioral symptoms starting late in life in GM2/GD2 synthase null mice, and only complete hearing loss in GM3 synthase knock-out mice [80,81]. Lack of motor symptoms and neurodegeneration in these latter mice might be explained by compensatory up-regulation of the 0-series pathway [82]. Such a compensatory mechanism, however, is not present in humans with the same genetic defect [83]. These considerations should invite to caution when extrapolating data in animal models to human pathologies. An extensive review on models of impaired sphingolipid synthesis and associated pathology has been published recently [80]. In the next section of this review we will discuss the involvement of SM, ceramide and gangliosides in aging and neurodegenerative disorders.

7. Sphingolipids in brain aging and neurodegeneration

The lipid profile of the brain changes during aging and in some neurodegenerative diseases. Most changes occur in specific brain areas or even at distinct brain membrane microdomains. The mechanisms responsible for lipid changes, as well as the implications of the altered lipid profile depend mostly on the disease, although some general mechanisms might exist. Aberrant conversion of SM to ceramide as well as pronounced changes in ganglioside patterns have been involved in the pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease[4] (HD) and Human Immunodeficiency Virus (HIV) 1-related dementia, among others [16]. Lipid-storage diseases characterized by impaired degradation and accumulation of sphingolipids have been reviewed elsewhere.

7.1. Aging

Some pivotal mechanisms in aging in all organs are enhanced oxidative stress, mitochondria dysfunction and calcium dysregulation. In addition, in the CNS there are dramatic changes in the endosomal/lysosomal system characterized by increased expression of lysosomal proteases and reduction of lysosomal membrane stability [12]. As presented above most processes involved in aging are affected by simple sphingolipids and gangliosides.

The role of ceramide in aging has been recently reviewed [84]. Briefy, during aging there is a progressive increase in brain ceramide in the mouse cerebral cortex and hippocampus, and enhanced levels and activity of SMase mainly in striatum and hippocampus of animal models of senescence. The evidence indicates that SMase activation is secondary to activation of the insulin/insulin-like growth factor 1 receptors (IR/IGF1-R), which would cause a switch in expression levels of neurotrophin receptors from TrkA to p75NTR [85]. p75NTR is responsible for activation of neutral sphingomyelinase (nSMase) and ceramide formation. Increase in SMase activity and in ceramide levels may contribute to the vulnerability of the striatum and hippocampus to stress and inflammatory insults that lead to synaptic dysfunction and neurodegeneration [86]. In particular, ceramide enrichment would make the cells more susceptible to oxidative stress, a key process during aging and the development of neurodegener-

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ative disorders. Reciprocally, ROS can induce the increase in ceramide.

Throughout life, the ganglioside pattern in the brain changes continuously, with decreasing proportions of GM1 and GD1a and increasing proportions of GD1b, GM3, and GD3. After 70 years of age the ganglioside concentration decreases dramatically and at a faster rate than cholesterol and phospholipids. The concentration of GD3 in cerebral cortex increases 75% from 20 years to old age [87]. The role of GD3 in aging might be linked to its effects on mitochondrial functions. Individual C-18 ganglioside species remain constant during aging, while C20-species of gangliosides increase, in particular GM1/GD1 in the hippocampus [4]. The interpretation has been that C20 species would decrease membrane fluidity, altering membrane properties and making the cells more prone to degeneration. In line with studies performed on brains, lipid rafts isolated from neurons aging in vitro have high content of sphingolipids, particularly ceramide, decreased levels of gangliosides, and show changes in the pattern of associated proteins [88]. The opposing direction in the changes of ceramide and gangliosides during development and aging has served as the foundation for the development of an interesting concept that predicts the progression from "glycosignaling domains" during development to "ceramide signaling domains" during aging [88]. However, changes in lipid composition of brain lipid rafts during aging are not uniform. Lipid rafts isolated from mouse brain synaptosomes get enriched in GM1 [89]. This enrichment is brain region specific, more pronounced in mice expressing human apoE4 (an important risk factor for AD), and resistant to the treatment with the cholesterol sequestering agent methyl-β-cyclodextrin [89]. During aging, GM1 accumulates in clusters only in synaptosomes, and not in other brain lipid rafts, possibly as the result of SM increase, which also takes place in synaptosomes exclusively. These changes have been linked to the development of Alzheimer's disease (see below) [90].

7.2. Alzheimer's disease

The role of ceramide and other lipids in AD has been recently reviewed [16,84], thus the reader is referred to this work for a detailed discussion of original papers.

Ceramide increases very early in the brain of AD patients. aSMAse activity is elevated in brains of AD patients when compared to healthy, age-matched brains [91], likely due to the membrane-associated oxidative stress characteristic of AD. However, the molecular species of ceramide in AD brains correlate better with sulfatide molecular species than with SM molecular species, and brain sulfatide levels are reduced.

In AD an interesting reciprocal regulation exists between sphingolipids and Aβ (Fig. 4A), the characteristic amyloidogenic peptide that accumulates in the brain. Sphingolipids regulate amyloid precursor protein (APP) cleavage and Aβ production by means of the localization of the two enzymes that cleave APP in the amyloidogenic pathway, γ-secretase and β-site APP-cleaving enzyme (BACE), to lipid rafts. SM reduction and ceramide stabilize BACE1 and promotes β cleavage of APP. Importantly, the mechanism of BACE1 stabilization appears independent of the formation of ceramide platforms but seems to result from a more direct regulation of BACE by ceramide and other lipids [92]. Lipid rafts are also important in neuronal uptake of Aβ. We have demonstrated that Aβ uses a raft-dependent endocytic pathway for internalization into neurons and that inhibition of sphingolipid and cholesterol synthesis significantly reduces neuronal uptake of Aβ [93]. Therefore, sphingolipids could regulate the level of intraneuronal Aβ, which is important for brain toxicity and synaptic plasticity abnormalities in AD.

The reciprocal aspect of sphingolipid functions in AD is represented by the role of ceramide as second messenger of Aβ cytotoxicity. Many mechanisms are responsible for ceramide accumulation downstream of Aβ [84].

The role of gangliosides in the pathology of AD has been recently reviewed [94,95], then we will only mention the main mechanisms by which gangliosides influence AD. In the brain of AD patients the pattern of gangliosides is significantly changed in a region- and ganglioside-specific manner. Gangliosides are involved in many important cellular processes that are linked to AD development, thus it is difficult to reach a verdict whether they are beneficial or detrimental.

Defective GSL synthesis decreases APP cleavage by α- and β-secretase by impairing the transport of APP through the secretory and endocytic pathway, and to the plasma membrane and endosomes where the secretases are located [92,96]. In agreement, studies in vitro demonstrated that administration of exogenous GM1 increases secretion of Aβ [97], suggesting that GM1 therapy would be detrimental for AD. Nevertheless, systemic delivery of GM1 to transgenic mice overexpressing APP causes a substantial decrease in the levels of soluble Aβ and a reduction of amyloid burden in the brain [98]. These apparent discrepancies can be reconciled if one considers the different route of delivery. When GM1 is provided directly to cells it will incorporate into the plasma membrane and will change membrane dynamics favoring the access of secretases to APP. On the other hand GM1 delivered systemically would bind to Aβ triggering a "sink" effect at the periphery without acting at the brain cellular level.

One of the most significant aspects of the role of GM1 and other gangliosides in AD results from their ability to bind Aβ [95] (Fig. 4A). Aβ binds to membranes containing GM1, and upon binding, it undergoes a conformational transition from random coil to an ordered structure rich in β-sheet. GM1 acts as seed for Aβ fibrillation in the brain (reviewed in [94]). The seeding process starts with an increase in GM1 at the plasma membrane, possibly as a consequence of abnormalities in the late endocytic pathway [99]. High-density GM1 clustering induces Aβ assembly in presynaptic neuritic terminals in the brain in an age-dependent, and partially brain-region-specific manner [90]. This particular localization of GM1 clusters is in line with evidence of Aβ deposition at the presynaptic neuritic terminals [94].

Other gangliosides also interact with Aβ causing Aβ accumulation in the brain. The assembly of wild-type and mutant forms of Aβ is accelerated in the presence of GM1, GM3 and GD3 gangliosides (reviewed in [94]. Moreover, elimination of GD3 synthase (GD3S) in a triple transgenic model APP/PSEN1/GD3S$^{-/-}$ improves memory and reduces Aβ plaque load [65]. These animals have decreased levels of GD3 but increased GM1 and GD1a, therefore it remains to be determined whether the effects observed were due to decreased GD3, increased GM1 or both.

A new role for gangliosides in AD has been recently proposed. The sialic acid moieties of gangliosides present in neuritic plaques in complexes with Aβ would serve as ligands for the receptor Siglec-11, a negative immune regulator expressed in microglia. By activating this receptor neuritic plaques would be able to evade the immune surveillance of microglial cells [100]. Sialylated glycoproteins present in neuritic plaques would play a similar role suggesting that plaque accumulation in AD could be exacerbated by glycosylation of amyloid aggregates and consequent down-regulation of microglia function.

7.3. Parkinson's disease

PD belongs to a group of conditions called synucleinopathies, which are characterized by the presence of fibrillary aggregates of α-synuclein protein in the cytoplasm of selective populations of neurons and glia. Hallmarks of PD are progressive degeneration of dopaminergic neurons in the substantia nigra and

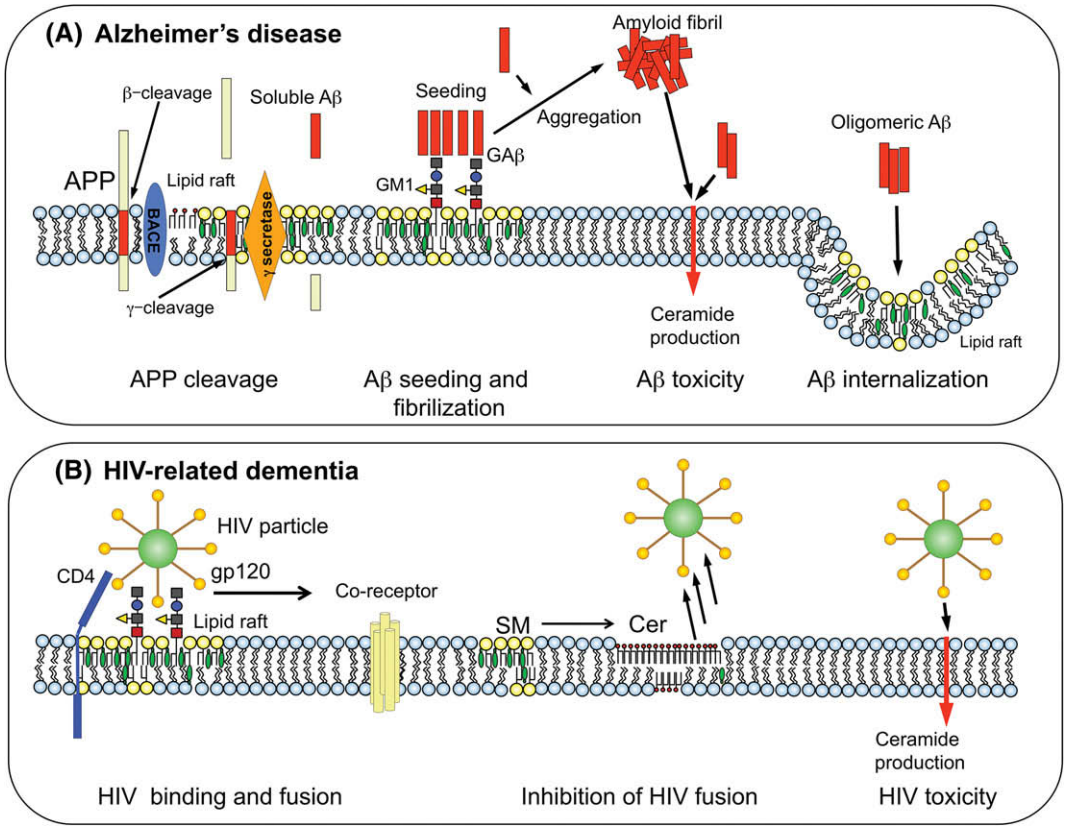

Fig. 4. Role of sphingolipids in AD and HIV-related dementia. (A) Localizaion of BACE1 and γ-secretase to lipid rafts enhances APP cleavage and production of Aβ. Ceramide has also a direct stimulatory action of BACE. The interaction of Aβ with GM1 at the plasma membrane induces Aβ fibrillation. Sphingolipids in lipid rafts are also crucial for Aβ internalization into neurons. Finally, Aβ increases production of ceramide by a variety of mechanisms, and ceramide mediates at least some of the cytotoxic effects of Aβ. (B) In HIV-related dementia lipid rafts help viral binding to the surface of target cells and viral fusion. GSLs binding to the viral protein gp120, promotes the interaction of the complex CD4/gp120 with the co-receptor CXCR and consequent viral fusion. Ceramide has both beneficial and detrimental roles. Increased production of ceramide blocks viral fusion. However, ceramide may also mediate viral toxicity.

subsequent dopamine deficits in the striatum. It is generally believed that oligomers and protofibrils of α-synuclein may play a causative role in neurotoxicity. Hence, inhibiting the aggregation of α-synuclein may constitute a rational therapeutic approach.

GM1 interacts with α-synuclein and inhibits its aggregation in vitro [101], raising the possibility that endogenous gangliosides might prevent α-synuclein aggregation in vivo. Gangliosides might also impair signaling mechanisms activated by α-synuclein by affecting the composition of caveolar membranes where α-synuclein is located. There is also extensive evidence of the beneficial effects of GM1 supplementation in animal models of PD [102]. Based on this evidence, it is conceivable that the elevated levels of anti-GM1 antibodies found in some PD patients would block normal GM1 function and could play a role in disease pathogenesis [103].

With respect to simple sphingolipids, in PD the conversion of SM to ceramide is affected (reviewed in [61]). There is a dramatic increase in SMase levels in the dopaminergic system and a differential regulation of aSMase versus nSMase. The oxidative stress that results from cathecolamines metabolism in dopaminergic neurons activates nSMase, causing an elevation of ceramide levels. Ceramide derived from SM might be responsible for the apoptosis observed in the substantia nigra, since a reduction of ceramide is protective against the specific dopaminergic toxin.

7.4. HIV-associated dementia

From the patients suffering from acquired immunodeficiency syndrome (AIDS), 20-30% present severe neurological disabilities identified as Human immunodeficiency virus type 1 (HIV-1)-associated dementia (HAD). HAD is linked to neuronal death but the exact mechanisms of HIV-induced apoptosis have not been completely elucidated. Interaction of the HIV-1 envelope glycoprotein gp120 with CD4 and the co-receptors CXC chemokine receptor 4 and 5 (CXCR4 and CCR5) present on the host cell surface, mediates viral fusion [16]. Lipid rafts play a major role in HIV1-fusion and consequently, their disruption by cholesterol depletion causes inhibition of HIV infection [104].

Various GSLs binds to gp120 through its SBD (see Section 2), galactoceramide with high affinity and GM3 and Gb3 with low affinity [104]. The interaction with GSLs helps stabilizing the virus at the cell surface, promoting the lateral motion of the complex CD4-gp120 to reach the co-receptor and aiding in the conformational changes of gp120 required for fusion (Fig. 4B). Therefore, the role of GSLs in HAD is mainly detrimental.

Ceramide may have both positive and negative effects on HAD. On one side, increasing ceramide biosynthesis makes cells resistant to HIV infection due to inhibition of membrane fusion, to the extent that the ceramide biosynthetic pathway has been proposed as a possible novel target for HIV treatment (reviewed in [16]) (Fig. 4B). On the other hand overproduction of ceramide may be involved in neuronal death in HAD patients [105]. There is evidence that an early dysfunction of sphingolipid metabolism leads to accumulation of ceramide in cerebrospinal fluid (CSF) of HIV patients with active dementia due to increased SMase activity, while higher levels of SM are present in CSF of HIV patients with inactive dementia [106]. Studies in cultured neurons have shown that gp120, induces the activation of SMase and generation of ceramide by coupling to the CXCR4 and the induction of NADPH

oxidase-mediated production of superoxide radicals. Based on this evidence, Jana and col. suggested that specific targeted inhibition of nSMase may be an important therapeutic approach to prevent neuronal damage in HAD [84].

7.5. Huntington's disease

Huntington's disease (HD) is an inherited neurodegenerative disorder determined by the expansion of a poly-glutamine (polyQ) stretch in the protein huntingtin (Htt). Misfolded mutant Htt (mHtt) forms intra-neuronal toxic oligomers and protein aggregates, resulting in the development of a broad array of cell dysfunctions, including transcriptional dysregulation, mitochondrial metabolism aberrations, and impairing cell signaling, axonal transport and synaptic activity. In addition, HD cells have increased susceptibility to stress and apoptotic stimuli.

We have recently demonstrated that levels of GM1 are lower than normal in cell and animal models of HD and in fibroblasts from HD patients.[5] Transcriptional dysregulation of the ganglioside biosynthetic genes was also recently confirmed in another model of HD [107]). Interestingly, our data suggest that levels of GM1 at the plasma membrane of HD cells correlate with cell susceptibility to apoptosis. Restoring normal ganglioside content in HD striatal cells by GM1 administration results in protection from cell death. Vice versa, decreasing GM1 in normal cells reproduces the susceptibility to apoptosis that is typical of HD. Therefore, GM1 at the plasma membrane of HD cells may play a role in the cell response to stress, perhaps by modulating the activation of pro-survival and/or pro-apoptotic pathways. Indeed, we have found that GM1 restores normal levels of active AKT, a pro-survival kinase the activation of which has been shown to be suboptimal in HD. In addition, GM1 administration leads to phosphorylation of mHtt, an event that is always associated to reduced mHtt toxicity and improved HD cell survival. Therefore, a ganglioside restorative therapy might be highly beneficial to HD patients.

8. Gangliosides and neuroprotection

The pharmacological use of gangliosides for neuroprotection has been tested since the late 80s. A plethora of studies in animal (including primates) and cell models has shown that administration of the ganglioside GM1 protects neurons from a variety of insults, ranging from mechanical injury to exposure to neurotoxins, growth factor deprivation and excitotoxicity (reviewed in [34]). How GM1-mediated neuroprotection is achieved in this vast diversity of experimental models is not completely understood, but most likely the mechanisms involved are multiple and different depending on the model used. The neuroprotective role of GM1 in models of excitotoxicity may stem from the ability of this ganglioside to regulate nuclear Ca^{2+} homeostasis [72]. In addition, GM1 might exert a neurotrophic function similar to neurotrophins, thanks to its ability to activate Trk receptors and downstream protective kinases [108]. Furthermore, GM1 administration has been shown to lead to increased transcription and release of NGF and neurotrophin 3 (NT-3), while a semisynthetic analogue of GM1, Liga20, induces release of BDNF (reviewed in [34]). Any of these general protective mechanisms would be beneficial in most neurodegenerative conditions. In the case of pathologies such as Alzheimer, Parkinson and Huntington's disease, in which the presence of toxic misfolded proteins or peptides is a common denominator of neural dysfunction, GM1 might activate additional protective mechanisms, such as stimulation of autophagy [70] or induction of fibrillogenesis and aggregation of toxic misfolded proteins, as described in Section 7.

In spite of the strong evidence of the neuroprotective effect of GM1 in animals and in in vitro models, early clinical trials in patients with stroke [109], Parkinson's disease [110] and spinal cord injury [111] have been disappointing or inconclusive at best. It is now evident, that at least some of these studies had flaws in the design or used GM1 doses that were 10–50 times lower than the therapeutic dose established for rats [109]. These considerations, together with the low permeability of the blood-brain barrier to GM1, might explain the overall lack of GM1 efficacy in clinical studies. GM1 analogues like LIGA-20, which is more prone to cross the blood-brain barrier, might have improved bioavailability and benefits [34], however, at least in certain cases its mechanism of action has been shown to be different from GM1 [112]. Continuous intracerebral administration of the ganglioside (for up to 1 year) was tested in a small number of early onset Alzheimer patients. Although the authors of that study reported a small improvement of cognitive functions, as well as improved mood and motor performance in the patients [113], a real benefit of the therapy could not be proved due to the small number of patients enrolled and the absence of appropriate controls. Nevertheless all these studies have demonstrated that GM1 administration in patients is safe and that adverse effects, such as the development of anti-GM1 antibodies leading to peripheral neuropathy, are rare [109]. Based on these considerations and on our data on the neuroprotective role of GM1 in HD, we believe that GM1 may represent a viable therapeutic approach especially in HD patients.

9. Concluding remarks

The impressive number of processes regulated by simple sphingolipids and gangliosides has proven that, as anticipated by their enrichment in the nervous system, these lipids have a pivotal role in neuronal function. As our understanding of the molecular events involving sphingolipids and gangliosides increases it becomes clear that general mechanisms at the membranes where these lipids reside do take place, but ultimately at least some specificity must exist to secure the appropriate cell response. Thus, the challenge to identify how the specificity is achieved in a particular context continues.

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