GANGLIOSIDES IN MEMBRANE ORGANIZATION

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ABSTRACT

Since the structure of GM1 was elucidated 55 years ago, researchers have been attracted by the sialylated glycans of gangliosides. Gangliosides head groups, protruding toward the extracellular space, significantly contribute to the cell glycocalyx, and in certain cells, such as neurons, are major determinants of the features of the cell surface. Expression of glycosyltransferases involved in the *de novo* biosynthesis of gangliosides is tightly regulated along cell differentiation and activation, and is regarded as the main metabolic mechanism responsible for the acquisition of cell-specific ganglioside patterns. The resulting sialooligosaccharides are characterized by a high degree of geometrical complexity and by highly dynamic properties, which seem to be functional for complex interactions with other molecules sitting on the same cellular membrane (*cis* interactions) or soluble molecules present in the extracellular environment, or molecules associated with the surface of other cells (*trans* interactions). There is no doubt that the multifaceted biological functions of gangliosides are largely dependent on oligosaccharide-mediated molecular interactions. However, gangliosides are amphipathic membrane lipids, and their chemico-physical,

aggregational and, consequently, biological properties are dictated by the properties of the monomers as a whole, which are not merely dependent on the structures of their polar head groups. In this Chapter, we would like to focus on the peculiar chemico-physical features of gangliosides (in particular, those of the nervous system), that represent an important driving force determining the organization and properties of cellular membranes, and to emphasize the causal connections between altered ganglioside-dependent membrane organization and relevant pathological conditions.

KEYWORDS

Gangliosides, membrane domains, lipid rafts, glycolipid-enriched membrane microdomains, liquid-ordered phase

CHEMICAL STRUCTURE OF GANGLIOSIDES

Gangliosides are glycosphingolipids (GSLs), i.e. amphipathic membrane lipids with oligosaccharide chains linked to the hydrophobic backbone, ceramide, responsible for the insertion in cellular membranes, via a β-glycosidic bond. Their nomenclature, as proposed by Svennerholm (1), is based on the structure of the oligosaccharide head groups. The most abundant gangliosides in mammals belong to the ganglio-series, and the ones from brain (the tissue with the highest ganglioside content) are mostly represented by structures belonging to the ganglio-4 series, characterized by the tetrasaccharide ß-Gal-(1-3)-ß-GalNAc-(1-4)-ß-Gal-(1-4)-ß-Glc-(1-1)-Cer. The signature sugar residue present in gangliosides, is sialic acid, which contains a carboxyl group. Ganglio-4 structures can bear one (“a-series”), two (“b-series”) or three (“c-series”) sialic acid residues linked to the internal galactose, and additional sialic acid residues on the external galactose (and rarely the N-acetylgalactosamine). Outside of the nervous system, the most abundant ganglioside in mammals is GM3, characterized by the trisaccharide α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc(1-1)-Cer.

Sialic acids (2)are derivatives of the 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, or neuraminic acid. Two main N-acylated forms of sialic acid are known to be present in natural gangliosides: the 5-*N*-acetyl-and the 5-*N*-glycolyl-derivative. Of the two derivatives, the former is the most abundant sialic acid in healthy mammals, including humans. About 10% of the total 5-*N*-acetyl-neuraminic acid (Neu5Ac) in gangliosides is *O*-acetylated (i.e., 5-*N*-acetyl-9-*O*-acetyl-neuraminic acid), and polysialogangliosides containing this sugar structure have been characterized in mice brains (3-5). Most mammals, including primates, are able to synthesize 5-*N*-glycolyl-neuraminic acid (Neu5Gc). Humans however are not able to do so due to the ancestral loss of the *CMAH* gene, encoding for the enzyme responsible for the conversion of CMP-5-*N*-acetyl-

neuraminic acid to CMP-5-*N*-glycolyl-neuraminic acid, in the hominid lineage. Minor amounts of gangliosides containing 5-*N*-glycolyl-neuraminic acid however are detectable in human tissues, likely due to the recycling of food-derivedNeu5Gc (6). This acid is also usually present at relatively high levels in gangliosides from certain tumors (7). Nevertheless, 5-*N*-glycolyl-neuraminic acid is almost absent in brain gangliosides (8). Polysialogangliosides in the form of lactones, with the sialic acids linked together with ketosidic and ester linkages, have been detected in fish, whale and human brain (9-11).

Gangliosides share the same hydrophobic moiety, ceramide, with all other sphingolipids (SLs). This moiety is composed of a long chain amino alcohol (12) (2*S*, 3*R*,4*E)* 2-amino-1,3-dihydroxy-octadec-4-ene, commonly known as sphingosine, linked to a fatty acyl chain via an amide bond. In mammalian gangliosides, the C18 molecular species of sphingosine is the most common, however, less frequent structures with either shorter and longer alkyl chain have been identified, and gangliosides containing C20 sphingosine are relatively abundant in mammalian nervous tissue and cultured neuronal cells (13). Further heterogeneity in the ceramide moiety is due to the presence of fatty acyl chains with different length and degree of unsaturation. The preference of the different known (dihydro)ceramide synthases for different acylCoA explains, at least in part, the different fatty acid composition of gangliosides and their tissue-specific composition from this point of view (14). Brain gangliosides are highly enriched in saturated acyl chains such as palmitic and stearic acid (15), and ceramide synthase 1 is consistently expressed at high level in human brain (16). Hydroxylated fatty acids, abundant in neutral glycolipids and sulfatides, are less common in gangliosides, however GM3 ganglioside from human liver contains a significant amount of α-hydroxylated C16-C24 chains, which remarkably increase withaging (17).

PHYSICOCHEMICAL PROPERTIES OF GANGLIOSIDES: A DRIVING FORCE FOR MEMBRANE ORGANIZATION

Life as we conceive it requires biological membranes, which provide the basic frame for the organization of every type of eukaryotic cell. Biological membranes separate the cellular and the extracellular environments and organize different compartments within cells, allowing the creation of gradients of water-soluble molecules (ions, enzymes, reaction substrates and products) and restricting the occurrence of specific biochemical reactions into specialized subcellular environments. The finding that the basic molecular organization of biological membranes is represented by a bilayer of amphipathic phospholipids is undoubtedly one of the milestones of modern cellular and molecular biology (18-20). The intrinsic properties of membrane lipids determine the fundamental structural and functional properties of biological membranes. In particular, the creation of the lipid bilayer, (which represents the first level of ordered organization of biological membranes) is lipid-driven, i.e., it is the consequence of the aggregational properties of complex amphipathic membrane lipids. Glycerophospholipids (GPL) are by far the major structural lipid components of eukaryote cellular membranes, and phosphatidylcholine (PC), that accounts in “average” membranes for more than 50% of all cell membrane phospholipids, is the main bilayer-forming lipid. A GPL bilayer as a whole is a very stable structure, in agreement with the primary function of cellular membranes as physical barriers. However, bulk membrane GPL are present in a large number of different molecular species. A large proportion of membrane phospholipids is rich in unsaturated fatty acids, ensuring that at physiological temperature the membrane bilayer is on a fluid, “liquid-disordered” phase, allowing a certain degree of lateral motility and conformational freedom to membrane components, including membrane-associated proteins. The behaviour of biological lipid bilayers as two-dimensional fluids under physiological conditions is the tenet of the “fluid mosaic” model proposed by Singer and Nicolson in 1972 (21). However, different kinds of lateral interactions among membrane components can take place, leading to the establishment of multiple levels of lateral order (including the organization of “liquid-ordered” domains(22)), and early observations

suggested that the membrane lipid components are not merely the solvent for membrane-associated proteins, but rather are active players in the creation of these levels of order. In fact, shortly after the fluid mosaic model had been formulated, studies of thermal effects in the behavior of membrane lipids suggested that phase behavior of lipid mixtures could be responsible for a certain degree of lateral organization in biological membranes (23-25). In 1982 the concept that the existence of multiple phases in the membrane lipid environment can drive the “organization of the lipid components of membranes into domains” (26) was clearly formulated. Heterogeneity of membrane lipids is the driving force for the lateral organization of biological membranes. If we focus on GPL, a significant amount of molecular species with less fluid, saturated chains is present. Lipids with a high content of saturated acyl chains (that possess a high melting point and are tightly packed with a high degree of order in the hydrophobic core of a bilayer) are characterized usually by a higher transition temperature, within or above the physiological range. The difference of transition temperature due to the different acyl chain composition probably represents one of the main forces leading to phase separation in lipid bilayers (27-29), as demonstrated by the observation that phase separation occurs in binary mixtures of PC molecular species differing in chain length and/or saturation (30-37).

Sphingolipids are ubiquitous but minor components of eukaryotic cell membranes, and in general complex glycosphingolipids, including gangliosides, are not bilayer-forming lipids. Gangliosides in water solution tend to form micellar aggregates (38,39), with the notable exception of GM3, which spontaneously aggregates as stable unilamellar vesicles (40). However, SL can be inserted in the GPL bilayer through their hydrophobic ceramide moiety. Even if minor membrane components, SLs and gangliosides are relatively abundant in some tissues or cell types. By far the most notable example is represented by the brain (41), where gangliosides are present at high levels in neurons (e.g., in cultured cerebellar neurons they represent about 5% of total amphipathic lipids, i.e., about 10% of total lipids of outer

membrane layer(42)) while sphingomyelin, galactosylceramide and its sulfated derivative, 3-*O*-sulfogalactosylceramide or sulfatide, are enriched in myelin-forming oligodendrocytes (43). In addition, there are striking differences in GSL content among different subcellular membranes, with the plasma membrane having the highest enrichment (44,45). Finally, within plasma membranes, GSL are asymmetrically enriched in the external leaflet, with their oligosaccharide chain protruding toward the extracellular space, where its sugar residues can engage *cis*– and *trans*- interactions with a wide variety of cell surface and extracellular molecules (46). Thus, the local concentration of GSL in specific cellular membranes can be relatively high, and their influence on the membrane organization can represent an important driving force for the creation of lateral order. It is worth to note that, even if the highest concentration of gangliosides is associated with the outer leaflet of the plasma membrane, consistent with the biological roles of oligosaccharide-dependent interactions mediated by gangliosides in the regulation of several aspects of the “social life” of the cell, ganglioside pools in other subcellular locations, e.g. the nuclear membrane, seem to be relevant(47).

SLs, and more specifically gangliosides, have several peculiar structural features that have important consequences in terms of the lateral organization of biological membranes. As discussed in the first part of this chapter, their molecular complexity is very pronounced: 1) the oligosaccharide chain of gangliosides is extremely variable due to the sugar structure, content, sequence and linkages, and is characterized by the presence of one or more negative charges due to the sialic acid residues; 2) ceramide, the double tailed hydrophobic moiety of gangliosides, is also heterogeneous in terms of chain length and presence of unsaturations.

The most remarkable feature of all gangliosides is the bulkiness of their hydrophilic head groups, as compared to that of phospholipids (Figure 1). The volume mismatch of the head groups of amphipathic lipids present in the same membrane represents the main

driving force for clustering and segregation of different membrane components, allowing minimization of the interfacial free energy. The area at the hydrophilic/hydrophobic surface of the membrane required to allow the insertion of the ganglioside molecule in the bilayer increases with the volume occupied by the head group. The larger the interfacial area required by ganglioside oligosaccharide structures, the more pronounced the segregation of ganglioside molecules within the phospholipid bilayer, and the more positive the curvature of the resulting ganglioside-rich membrane microenvironment. Thus, the volume of the sugar head groups directly determines the level of phase separation and the geometry in a specific membrane microenvironment. Published evidence clearly showed that gangliosides undergo phase separation in two-components, two-phases as well as in one-component PC bilayers (30-36), and in sphingomyelin bilayers (48). Moreover, phase separation has been observed in mixed micelles of two different gangliosides (GM2 and GT1b (49), GD1b and GD1b-lactone (50), or GM1 and GD1a (51)) with identical composition of the hydrophobic moiety (Figure 2).

A number of studies on semi-synthetic gangliosides with defined composition of the hydrophobic moiety, in monomeric form or inserted in model membranes (mainly phospholipid liposomes and single-component or mixed micelles) allowed elucidation of the contribution of different factors to the volume of the head groups (reviewed in (29)). Obviously, the volume occupied by the head group increases with the complexity of the oligosaccharide chain, and the extent of ganglioside phase separation depends on the number of sugars in the oligosaccharide head group (31,32). However, at least three additional factors are very relevant for the saccharide head groups: 1) the geometry and dynamics of the different glycosidic linkages present in the oligosaccharide chain; 2) the presence of the negative charge(s) of the sialic acid residue(s); and 3) the amount of hydration water associated with the oligosaccharide chains.

Molecular dynamics and high resolution NMR studies (52-60)(in particular carried out for the ganglio-series gangliosides, analysing the effect of the transition in the oligosaccharide chains (LacCer→GM3→GM2→GM1→GD1a/b→GT1b)) showed that the addition of a single sugar to a given oligosaccharide chain has very dramatic consequences, giving rise to very different inter-residual interactions within the oligosaccharide chains and generating very different dynamics for the glycosidic linkages even in very similar oligosaccharide structures. The result is that the number of possible conformations for very similar oligosaccharide chains differ substantially. The number of possible conformers of a given ganglioside oligosaccharide moiety directly influences the volume occupied by the ganglioside monomers at the membrane surface. The dynamic properties of the different glycosidic linkages in the oligosaccharides of GM2, GM1 and GD1a allow the existence of one, two and four preferred conformations, respectively. Thus, the volume required to accommodate the GM1 and GD1a oligosaccharides (characterized by a higher number of preferred conformations) is much higher than the volume that could be predicted based on the features of the GM2 oligosaccharide, simply considering the addition of one or two more sugars, respectively.

In the GD1b tetrasaccharide -β-GalNAc-(1-4)[α-Neu5Ac-(2-8)-α-Neu5Ac-(2-3)-]-β-Gal-, geometrical constraints led by interactions between GalNAc and the external Neu5Ac determine the arrangement of the tetrasaccharide chain in a circle with a diameter of 3 Å, with a central hollow characterized by a highly hydrophobic internal surface, apparently wide enough to accommodate cations (61). This peculiar conformation confers a bulkier character to the oligosaccharide chain portion in the proximity of the hydrophobic-hydrophilic interface. Consequently, GD1b bears a significantly larger surface area than its isomer GD1a.

In ganglioside lactones, e.g. in GD1b-lactone (9), the presence of the inner ester between the external sialic acid carboxyl group and the inner sialic acid residue, confers a rigid conformation to the trisaccharide -β-GalNAc-(1-4)[α-Neu5Ac-(2-3)-]-β-Gal-,which leads

to a better lining up of the disialosyl chain with the neutral oligosaccharide chain, reducing the angle between the neutral chain and the inner sialic acid axis (61). Consequently, the geometry of GD1b-lactone is closer to that of GD1a than to that of GD1b. This implies that the lactonization-delactonization process (occurring for example due to local pH changes in the proximity of the cell surface) might have very deep influence on the aggregational properties of gangliosides.

As mentioned above, the volume of the sugar head group is also influenced by the presence of hydrating water associated with the oligosaccharide chain. The amount of water associated with the polar head groups, due to the strong hydrophilic character of the oligosaccharide chains, is remarkably high (62). The number of water molecules tightly associated with a given oligosaccharide chain ranges from 22 to 60, depending on the structure of the oligosaccharide (while only 6-7 water molecules are associated with the phosphocholine head group of dipalmitoylPC) (63). NMR studies showed that the interactions between water molecules, the sugar residues sialic acid and inner galactose in the oligosaccharide of GM1 (58) are very strong. The contribution of the associated water to the volume of the sugar head group is confirmed by calculations performed on GM2 molecules, indicating a difference of about 5Å between the dry and hydrated micellar radius (49). Moreover, stable removal of water from the hydrophilic head of GSL in micellar dispersion lead to changes of the surface area occupied by the monomer inserted into the aggregate with consequent changes in surface curvature and in aggregate size (64-66). The presence of water has two other consequences on the properties of the ganglioside oligosaccharide chains, which reflect on their aggregational behavior as well as on their ability to engage *cis*- and *trans*- interactions with other molecules: 1) the associated water is likely relevant in avoiding repulsive effects between the negatively charged oligosaccharide chains, e.g. in ganglioside-rich membrane microdomains; moreover, a network of water-mediated hydrogen bridges could significantly contribute to the

stabilization of a glycolipid membrane clusters; 2) on the other hand, the presence of a thick and strongly associated water layer suggests that a direct role of carbohydrate-carbohydrate interactions in mediating ganglioside-dependent biological events is very unlikely (67).

In addition to the features of the hydrophilic portions, we have recalled the importance of the differences in the transition temperatures due to the different acyl chain composition of membrane lipids as one of the main forces leading to phase separation in lipid bilayers, and of the high transition temperature of the hydrophobic chains of gangliosides in the segregation process. Lipids with a high content of saturated acyl chains (that possess a high melting point and can be tightly packed with a high degree of order in the hydrophobic core of a bilayer) are characterized usually by a higher transition temperature, within or above the physiological range (29,68). Indeed, phase separation of sphingomyelin in dimyristoylPC bilayers depends on the degree of sphingomyelin chain mismatch (69), and the lateral phase separation of GM1 in PC bilayers(34) is directly correlated with the acyl chain length and inversely correlated with the degree of unsaturation. In general, for a given oligosaccharide composition, the extent of ganglioside lateral phase separation depends upon the length and unsaturation difference between the ganglioside long-chain base and PC acyl chains (30-32). It is important to recall that gangliosides containing saturated acyl chains, such as palmitic and stearic acid, are very abundant in some tissues, especially in the nervous system. Brain gangliosides are typical liquid-ordered phase lipids. In the liquid-ordered phase, a further stabilization element is represented by the enrichment in cholesterol, which can tightly intercalate between the ordered acyl chains of acyl-lipids (29,70). The role of cholesterol in driving/stabilizing liquid-ordered phase formation in biological membranes has been very much emphasized (71). Some studies in monolayer membrane models suggested the existence of a strong, some what specific lateral interaction between cholesterol and sphingomyelin, leading to the formation of liquid-condensed cholesterol- and sphingomyelin-rich domains (72-74). However, studies in phospholipid bilayers seem to confute this

evidence (75). On the other hand, cross-linking of GM1 ganglioside using pentavalent cholera toxin at 37°C in a relatively physiological model system (plasma membrane spheres obtained by a cell-swelling procedure from A431 cells) (76) resulted in the cholesterol-dependent coalescence of GM1-rich domains with the formation of micrometer-scale domains, characterized by a lower translational diffusion, and higher degree of lateral order (77).

In addition to the effect of the fatty acyl chains of GSL on their transition temperatures, the contribution of the part of the ceramide molecule sitting at the water lipid interface seems to be very relevant to the aggregation/segregation of these lipids within membranes. This is suggested by the increased order of C18-sphingomyelin molecules inserted into a dioleylPC bilayer due to the formation of sphingomyelin nanoclusters stabilized by hydrogen bonds (78,79). Moreover, SL-rich domains can be formed in cells that contain sphingomyelin but not GSL (80) and in subdomains enriched in sphingomyelin but with no gangliosides and a very low content of neutral GSLs (81,82). The player here is the amide group of ceramide at the water/lipid interface, which behaves as a rigid system in a planar conformation, together with the hydroxyl group in position 3 (83,84). The availability of an amide nitrogen, of a carbonyl oxygen and of a hydroxyl group, enables ceramide to act as hydrogen bond donor and acceptor at the same time. Therefore, this feature allows SL to form a stable net of interactions, which, in the case of gangliosides, further stabilizes the clustering driven by the properties of the hydrophilic head groups.

LOCAL MODULATION OF MEMBRANE GANGLIOSIDE COMPOSITION: A MECHANISM FOR THE FINE TUNING OF MEMBRANE LATERAL ORGANIZATION

The pattern of gangliosides expressed at the plasma membrane of a given cell type, and thus the resulting ganglioside-driven membrane organization, is specific and tightly regulated along development and dependenton the functional status of the cell. The genetic and epigenetic mechanism underlying this regulation have been particularly well studied in

the nervous system (85-87), not surprisingly considering the high enrichment of gangliosides in neurons. However, it is clear that, even for cell populations expressing relatively low amounts of gangliosides (e.g. immune system cells such as T cells), the activation of different cellular subpopulations requires the expression of distinct ganglioside patterns (88). In addition, even in the well-studied nervous system, the availability of analytical techniques with high spatial resolution such as imaging mass spectrometry are revealing an amazing heterogeneity in ganglioside pattern among different cellular populations and substructures. For example, it has been reported that ganglioside distribution in the hippocampus is different in the three layers that constitute the molecular layer of the dentate gyrus, indicating a strikingly different expression of gangliosides in adjacent neuronal subpopulations and in neuronal axons *vs.* dendrites (89).

The regulation of plasma membrane ganglioside composition is mainly dependent on the biosynthetic and catabolic processes occurring in intracellular districts (90), thus the turnover of plasma membrane gangliosides is intimately connected with a bidirectional traffic, from and to the plasma membrane, that mainly occurs via vesicular transport, even if non vesicular transport via sphingolipid binding proteins plays an important role in specific steps (91). On the other hand, several enzyme activities potentially involved in the local remodeling of SL composition are associated with the plasma membrane, providing the machinery for fast alterations of membrane organization in response to different stimuli. This concept was originally proposed for the interconversion of sphingomyelin into ceramide. Different sphingomyelinases are resident at the plasma membrane level or can be translocated to the plasma membrane from intracellular sites. In addition, at least one isoform of sphingomyelin synthase is enriched in the plasma membrane. Thus, the plasma membrane ratio between ceramide and sphingomyelin can be effectively regulated at the local level. Ceramide has important roles as a bioactive molecule, acting on plasma membrane-associated and intracellular targets. In addition, ceramidases and sphingosine

kinases are also associated with the plasma membrane, thus hydrolysis of sphingomyelin has been regarded by many authors mainly as a mechanism to generate bioactive ceramide and/or sphingosine 1-phosphate (92). However, the sphingomyelin/ceramide interconversion at the plasma membrane has potentially important consequences on the membrane organization, strongly influencing membrane curvature and lateral organization. In particular, reorganization of lipid membrane domains into ceramide-rich signaling platforms has been reported to occur upon different receptor-dependent and -independent stimuli (93,94). An increasing body of evidence indicates that active plasma membrane metabolic remodeling also occurs for GSL (Figure 3). It has long been shown that synaptosomal membranes, a subset of neuronal membranes highly enriched in gangliosides, carry both a sialidase (95-98), and a sialyltransferase (99) activity. The existence of a ganglioside-sialidase activity at the plasma membrane was further suggested by enzymatic and immunological studies (100-103), and by metabolic studies in intact cells as well. Cultured rat cerebellar neurons and human neuroblastoma cells are able to desialylate exogenously added gangliosides under experimental conditions, preventing ganglioside internalization and/or blocking lysosomal function (104,105), and a membrane-bound sialidase was purified from human (106) and from bovine brain (107). Finally, the existence of a specific membrane-linked sialidase, distinct from other known sialidases, has been proven by the group of Miyagi, who cloned the cDNA sequence for human (108), bovine (109) and mouse (110) plasma membrane-associated sialidase, later named Neu3 (111). Following studies suggested that this enzyme plays a crucial role in modifying the cell surface ganglioside composition, in particular causing a shift from polysialylated species to GM1, a decrease of GM3 and a parallel increase in lactosylceramide, with deep consequences on very important cellular events, such as neuronal differentiation and apoptosis in colon cancer (112). Neu3 is not randomly distributed at the cellular surface, but rather it is targeted to and dynamically associated with detergent-insoluble, GSL-enriched

microdomains (113-116), suggesting that membrane topology is highly favoring the accessibility of the putative substrates to the enzyme activity. On the other hand, several experimental data show that the local activation of Neu3 might represent an efficient mechanism to regulate the local ganglioside composition of specific membrane subdomains, thus affecting the biological activity of signaling proteins resident in or recruited to these membrane domains. In other words, the most relevant biological effects of Neu3 seem to be linked to its ability to modulate the composition of ganglioside-driven signaling units. From this point of view, the most striking example is represented by the ability of Neu3 to locally increase the surface concentration of GM1 in neurons, thus affecting the biological activity of the TrkA neurotrophin receptor. The physiological mechanisms responsible for Neu3 activation remain to be elucidated, however it has been recently shown that phosphatidic acid, produced via epidermal growth factor (EGF)-induced activation of phospholipase D, promotes the activation and surface localization of Neu3 (117). It has long been known that ganglio-series gangliosides, and in particular GM1, possess neuritogenic and neuronotrophic properties (118,119), exerting positive effects on neuronal growth, differentiation and survival both *in vivo* and in cultured neurons, and a protective effect against neuronal injury (120-123). Thus, GM1 seems to potentiate or to replace neurotrophins in their action on neuronal cells (124), and the underlying molecular mechanism is likely an effect on the activation of neurotrophin receptors. GM1, which is able to stimulate Trk receptor kinase activity, receptor autophosphorylation and dimerization, specifically and tightly binds Trk family receptors in cultured cells, brain tissue and *in vivo* (recently reviewed in (125)). A significant pool of Trk receptors is detergent-insoluble and associated with lipid rafts under basal conditions (126-131), moreover Trk receptors can be recruited to detergent-insoluble membranes from other cellular compartments upon stimulation (126,127). In addition, the glycosylation of Trk is required for the formation of Trk-GM1 complexes and for the targeting of Trk into GM1-enriched domains (132). This

suggests that lateral interactions between the oligosaccharide chains of the receptor and of GM1, mediating the receptor localization within ganglioside-rich membrane areas, could be a relevant mechanism for the regulation of its function. From this point of view, the activity of Neu3 in modulating the local membrane ganglioside composition seems to be crucial. The artificial modulation of Neu3 expression, in a positive or negative sense, deeply affects neuritogenesis (133,134). In addition, in cultured hippocampal neurons, the activity of the plasma membrane-associated ganglioside sialidaseaffects the local GM1 surface levels, and is essential for axonal growth and regeneration after axotomy (135). In these cells, Neu3 activity is asymmetrically concentrated at the end of one single neurite, and determines the neurite's axonal fate by the rearrangement of GM1 TrkA microdomain with a consequent local increase in TrkA activity (136).

Interestingly, Neu3 overexpression in cultured skin fibroblasts was able to modulate the production of bioactive ceramide at the cell surface, providing the first direct evidence of a link between glycosphingolipid metabolism and ceramide-mediated signaling, and suggesting that ganglioside hydrolysis, in addition to sphingomyelin hydrolysis, might contribute to the formation of ceramide-rich membrane platforms (137). Neu3-assisted cell surface ceramide generation from ganglioside GM3 indirectly demonstrate the presence of the other two active glycosylhydrolases, β-glucosidase and β-galactosidase, in the same plasma membrane district. Subsequently, several enzymes involved in GSL catabolism have been found associated with the external leaflet of the cell plasma membrane: β-hexosaminidase, β-galactosidase, β-glucosidase GBA1 and GBA2(138-141).

These observations open the possibility that extensive hydrolytic remodeling (not only desialylation) of membrane gangliosides can occur and contribute to the local membrane glycolipid composition (Figure 3). In addition, *in situ* sialylation of gangliosides also occurs at the cell surface. The existence of a synaptosomal membrane sialyltransferase in calf brain (99) has been confirmed by metabolic studies in chicken embryos (142) and rat brain

(143,144). Moreover, dexamethasone treatment in mouse thymus markedly increased GM3 synthesis, due to increased enzyme activity of GM3 synthase at the plasma membrane (145), thus confirming that glycolipid sialylation might occur outside the Golgi compartment, contributing to the local modulation of cell surface glycolipid patterns.On the other hand, glycosylation/deglycosylationare possible mechanisms for the local regulation of the plasma membrane GSLcomposition and lateral organization. As mentioned above, some gangliosides do contain ester linkages, which are unstable under alkaline conditions (while the majority of gangliosides, lacking this kind of linkage, are stable under alkaline conditions). The so-called “alkali-labile” gangliosides have been detected in mammals, birds, reptiles, amphibians, teleost, elasmobranches and cyclostomes (146), and in particular they are present in the ganglioside mixtures extracted from the animal brains. In some cases, the amount of alkali-labile gangliosides can be around 10% of total brain gangliosides, thus they are not negligible membrane components at least in the nervous tissue. There are two main types of alkali labile gangliosides: ganglioside lactones and ganglioside containing *O*-acetylated-sialic acid. In both cases, the alkali-labile species are more hydrophobic than the corresponding alkali-stable parent structure, thus, their conformational/aggregational properties are significantly different (see above for the detailed discussion of the conformation of GD1b-lactone).

Ganglioside lactones are formed by a reaction between the carboxyl group of sialic acid and one of the hydroxyl groups available in the oligosaccharide structure. The most stable reported lactonic linkage is inside the disialyl residue, Neu5Ac-(2-8,1-9)-Neu5Ac-. Ganglioside lactones remained elusive for long time, due their possible artifactual formationat acidic pH during the extraction procedures. Under acidic pH, the disialyl residue of GD1b ganglioside spontaneously undergoes an equilibrium reaction between the two molecules that contain the Neu5Ac-(2-8)-Neu5Ac (GD1b) and the Neu5Ac-(2-8, 1-9)-Neu5Ac (GD1b-lactone) disialyl residue, respectively (147). The existence of the

ganglioside lactones in mammalian brains was originally established by two-dimensional TLC analyses by treating the plate with ammonia vapors before the second chromatographic run (146,148,149). Under these conditions, the lactone is converted into the corresponding amide derivative that can be clearly separated in the second TLC run. The structure of GD1b-lactone extracted from human brain was subsequently characterized by mass spectrometry. Remarkably, the content of GD1b-lactone in the human brain progressively increased with age (9). *In vivo*, GD1b seems to be the natural precursor of GD1b-lactone in brain. Intracisternal injection of tritium-labeled GD1b in rats led to a progressive increase of tritiated GD1b-lactone in the following three days. Then tritiated GD1b-lactone levels decreased together with tritiated GD1b due the membrane turnover and ganglioside catabolic pathway. This suggests that the lactonization process occurs at the cell surface(150).

Ganglioside lactones are extremely resistant to the action of bacterial sialidase (150). Based on the knowledge about the sensitivity of gangliosides to sialidase hydrolysis, this suggests that lactones should be resistant to the action of cell surface sialidase Neu3. This would imply that the lactonization process might be important in regulating the ability of Neu3 to modulate the production of GM1 from GD1b. GD1b and GD1b-lactone have opposite effects on phospholipase C (PKC)-mediated protein phosphorylation (151). PKC is present in a ganglioside-rich membrane microenvironment, and it has been reported that its activity is modulated by changes in ganglioside membrane organization (152). Thus, the equilibrium between ganglioside GD1b and its lactonic derivative could be instrumental for membrane reorganization and the concomitant regulation of protein phosphorylation by PKC.

O-Acetylated sialic acid is component of both glycoproteins and glycolipids. Sialic acid can be *O*-acetylated in position 4, 7, 8 and 9. Three different sialate-*O*-acetyl transferase (SOAT) showing different position specificity have been identified (153). Sialic acid

acetylation occurs on the CMP-Neu5Ac before the sialic acid is transferred to the glycan acceptor by a sialyltransferase (153).

Human brain gangliosides contain about 10% of *O*-acetylated Neu5Ac, with respect to the total sialic acid (154). *O*-acetylation introduces a further element of great heterogeneity in the structure of ganglioside oligosaccharide chains, whose consequences are still poorly understood but potentially of great biological relevance. Two polysialylated *O*-acetylated gangliosides were purified and characterized from mouse brain, GT1b and GQ1b with a Neu5,9Ac_2 as external sialic acid of the disialyl residue linked to the internal galactose (4,5). Other *O*-acetylated gangliosides were extracted from cells and characterized: *O*-acetylated-GD3, with Neu5,9Ac_2 as external sialic acid, from melanoma cells (155), and two different *O*-acetylated-GM3, containing Neu5,4Ac_2 (156) and Neu5,4,9Ac_3 (157), from erythrocytes. In addition, *O*-acetylated GD2 and GT3 gangliosides were reported as components of a plethora of normal and pathological tissues and cells (153,158,159). The levels of *O*-acetylated gangliosides are relatively high in tissues characterized by a marked cell proliferation, suggesting that they might be markers for this biological condition. One of the most reported *O*-acetylated gangliosides is the *O*-acetylated-GD3, which is regarded as an oncofetal marker in many animal and human tumors (see as example (160)).

As mentioned above, information on the functional role of nervous system *O*-acetylated gangliosides is scant and fragmentary. The nervous system of normothermic dormice (*Glis glis*) contains alkali-labile gangliosides many of which are *O*-acetylated gangliosides. They were present in all examined area ranging from about 10% in olfactory bulb to 30% in spinal cord. However, in hibernating dormice animals *O*-acetylated gangliosides were found only in spinal cord, where they covered only 3% of total gangliosides (161). For some ganglioside species, the disappearance of *O*-acetylation in hibernating animals was paralleled by an increase of the corresponding alkali-stable counterparts, suggesting a different balance between SOAT and *O*-acetylhydrolases activity/expression in the two life conditions.

However, this was not true for all alkali-labile species, suggesting that different Golgi biosynthetic processes also occur. In addition, an increase of GM1 ganglioside, which was never detected as the O-acetylated species, was observed in hibernating animals. This suggests that the adaptation of the animals implies a deep rearrangement and organization of the plasma membrane mediated by gangliosides, whose biological significance remains to be elucidated.

CHANGES OF GANGLIOSIDE-DRIVEN MEMBRANE ORGANIZATION AS A PATHOGENETIC MECHANISM

If changes in the plasma membrane ganglioside composition could represent a physiological mechanism for the regulation of cellular functions, which are dependent on the organization of the membrane microenvironment of signaling proteins, the obvious implication is that alterations of ganglioside expression associated with diseases might lead to abnormal membrane organization, which could contribute to the pathogenesis of the disease. Anomalies in gangliosides metabolism and/or traffic, leading to altered gangliosides patterns, have been reported in an incredible number of different pathological conditions. However, cancer, neurodegenerative diseases and, more recently, diabetes are the diseases where a causal connection between altered ganglioside expression and consequent alterations in ganglioside-driven membrane organization as a relevant pathogenic mechanism seems to be convincingly proven. In this section, we will provide some examples with the aim to elucidate the molecular basis of this connection.

“Aberrant glycosylation” has been described for over 4 decades in almost all types of experimental and human tumors (162-164). Even if altered expression of glycosylated epitopes in cancer is not restricted to GSL, ganglioside-dependent changes of membrane organization play a major role as determinants for aggressiveness and invasiveness of human cancer. Particular emphasis in this regard has been posed on the role of altered sialylation of glycolipids, and the role of gangliosides in regulating the function of membrane-

associated signaling complexes, thus affecting tumor cell growth, survival, motility and invasiveness, has been described in detail in the last 15 years. The clinical relevance of these observations has been highlighted by the finding that the expression of two of the main metabolic enzymes whose activity controls the sialylation levels of gangliosides, sialidase Neu3 (165) and GM3 synthase (*St3gal5*) (166), is deeply altered in tumor tissues, suggesting that these metabolic enzymes could represent potentially druggable targets for novel therapeutic opportunities at least in some cancer types.

Growth factor receptor-dependent cell proliferation is controlled and modulated by gangliosides. In particular, EGF receptor (EGFR) autophosphorylation is subjected to inhibition by GM3 (167), due to a highly specific ganglioside-receptor interaction (167,168) involving side-by-side carbohydrate-carbohydrate interactions between the sialyllactose chain of GM3 (168) and a N-linked glycan bearing multiple GlcNAc terminal residues on the receptor (169,170). On the other hand, in addition to this specific GM3/EGFR interaction, GM3-driven membrane organization is an important additional factor in the regulation of EGFR activity. GM3/EGFR interaction is facilitated by the enrichment of EGFR in ganglioside-enriched, Triton X-100 insoluble membrane domains (171,172). On the other hand, caveolae and caveolin-1 are involved in the modulation of EGFR signalling (173,174), and caveolin membrane dynamics are deeply affected by gangliosides (175). EGFR is localized within a caveolin-rich fraction in A431 cells, however, EGFR-containing membrane domains can be separated from caveolae (176,177), suggesting a dynamic equilibrium between different membrane-associated EGFR pools. In a keratinocyte-derived cell line, GM3 overexpression induced a shift of caveolin-1 to EGFR-rich membrane regions, allowing its functional interaction with the EGFR receptor, that caused inhibition of EGFR tyrosine phosphorylation and dimerization (178). Thus, increased membrane GM3 levels influence EGFR signaling by a second distinct molecular mechanism, modulating EGFR/caveolin-1

association. Moreover, GM3-rich membrane domains regulate the cross-talk of EGFR with integrin receptor signaling: in the presence of high membrane levels, the interaction of integrin β1 subunit with EGFR and the consequent signaling cross-talk is perturbed (179).

Independentof its cross-talk with EGFR signaling, integrin receptor function is heavily regulated by the association with ganglioside-rich membrane domains, and this regulation is likely the most relevant molecular mechanism underlying the effect of gangliosides on tumor cell motility and invasiveness. The term “glycosynapse” has been introduced by S. Hakomori (180-182) to describe a ganglioside-rich membrane domain involved in the regulation of carbohydrate-dependent cell adhesion and motility. Glycosynapses are non-caveolar, cholesterol-independent ganglioside-rich membrane domains (183), characterized by the presence of tetraspanins (CD9, CD81, CD82) highly hydrophobic integral membrane proteins with four transmembrane stretches. Tetraspanins strongly interact with gangliosides in glycosynapses (184) and serve as the molecular pivot for the organization of membrane signaling complexes, whose function is in turn regulated by the levels of gangliosides in the membrane microenvironment. In particular, tetraspaninsare associated with and regulate the function of integrin receptors (185), involved in the regulation of tumor cell motility/invasiveness. Tetraspanin CD9 and integrin α3 or α5 receptor subunits are co-localized in glycosynapses. In a non-invasive bladder tumor cell line (originated by superficial, non-metastatic human bladder cancer), characterized by high levels of GM3 ganglioside, CD9/integrin association is positively modulated, and a multimolecular complex between GM3, tetraspanin and integrin α3β1 or α5β1 is stabilized (186-188). In the presence of the GM3-dependent integrin/CD9 complex, the c-Src kinase Csk, responsible for the phosphorylation of c-Src at Tyr527, is translocated to the GM3-rich membrane domain with consequent c-Src inactivation (188). Thus, the formation of integrin/CD9/GM3 complexes leads to the negative regulation of integrin-mediated cell

adhesion and tumor cell motility. Conversely, in highly invasive bladder cancer cells, characterized by lower levels of GM3, the integrin/CD9 association is reduced and c-Src is highly active, leading to reduced adhesion and increased motility. A similar role in the stabilization of an integrin/tetraspanin complex has been suggested for GM2, with consequent regulation of Met tyrosine kinase activity (189).

GM3-rich glycosynapses are involved also in the control of other forms of tumor cell adhesion, for example in adhesion mediated by GSL-GSL interactions. In the case of GM3-dependent adhesion of melanoma cells to the neutral glycolipid Gg3, it has been shown that GM3 is closely associated with c-Src, Rho and Ras within glycosphingolipid-enriched membrane domains and binding with Gg3 or anti-GM3 antibody stimulates focal adhesion kinase phosphorylation and c-Src activity (81). A similar GM3-dependent association between c-Src and other related signaling molecules was observed also in neuroblastoma cells (190).

In other tumors, regulation of Src-dependent tumor cell motility by ganglioside-rich signaling complexes might occur thanks to molecular mechanisms not involving tetraspanins.We showed that human ovarian carcinoma cells, characterized by low *St3gal5* expression, GM3 synthase activity and ganglioside content, are highly motile. In clones with increased GM3 synthase expression, obtained by *St3gal5*gene transfection (associated with an overall increase in cellular ganglioside levels), the *in vitro* motility was strongly reduced. GM3 synthase overexpression was paralleled by a marked overexpression of another hydrophobic membrane adapter protein, caveolin-1 (191). Caveolin-1 is supposed to concentrate signaling molecules within cholesterol- and sphingolipid-rich membrane domains, and the effect of caveolin-1 on tumor phenotype seems to be very heterogeneous and strongly dependent on the molecular partners interacting with this protein (192). In highGM3 synthase, high caveolin-1 cells, caveolin and gangliosides are tightly interacting together forming a detergent-insoluble membrane complex (193). Integrin receptor subunits

and the non-receptor tyrosine kinase c-Src are associated with the ganglioside/caveolin-1 complex. Caveolin-1 (175,194) and Src kinases (195,196) are associated with sphingolipid-enriched membrane domains also in other cell types, and it has been suggested that caveolin-1 might act as a membrane adapter coupling integrin receptors to Src kinases (197), and that caveolin-1-mediated inactivation of the integrin/Src/FAK pathway might be responsible for the inhibition of metastatic potential in melanoma (198). Our results strongly support the hypothesis that the inactivation of c-Src by a ganglioside/caveolin-1 complex might be linked to the downregulation of ovarian carcinoma cell motility.

Complex alterations in brain lipid composition have been reported along with physiological ageing, including reduction of total lipid content, decreased ganglioside content, and altered sphingoid base compositions in SL (199). These changes have deep consequences on the physicochemical properties of lipid rafts (for example, on local membrane microviscosity), which seems to change with the decline of physiological performance of the brain along ageing. In other words, lipid-dependent membrane organization naturally undergo an "ageing" process. In addition, alterations in membrane thermodynamics are more pronounced in mouse models of dementia (200,201). Congenital disorders of sphingolipid metabolism, such as gangliosidosis, due to defects in the lysosomal degradation of gangliosides, some forms of Gaucher disease, due to the defective activity of lysosomal glucosylceramidase, and Niemann Pick disease type A, due to the lack of acidic sphingomyelinase, are progressive and severe neurodegenerative diseases (reviewed in (202,203)). These diseases are hallmarked by accumulation of the undegraded SL at the lysosomal level, as the consequence of the enzymatic defect. The accumulated SL are different depending on the specific defect, however in all of the diseases traffic of lysosomal membranes with anomalous sphingolipid composition toward the plasma membrane (204) seems to have important consequences on the plasma membrane lipid composition and organization (202,205-207). In addition, secondary alterations in

ganglioside metabolism, likely due to extra-lysosomal events, have been reported in many of these diseases (208).

However, alterations in the metabolism of gangliosides possibly impacting on membrane organization have been reported for several important nervous system diseases, including most of the neurodegenerative diseases and the major forms of dementing disease, even in the absence of genetic defects directly related to sphingolipid traffic and/or metabolism (202,209). We will limit our discussion to Alzheimer's disease (AD), which in our opinion represents the most complex and intriguing example of the consequences of altered ganglioside-driven membrane organization in nervous system pathology.

Dysregulated brain ganglioside metabolism has been reported in brain of AD patients and in transgenic mice models of the disease (reviewed in (210)). The patterns of ganglioside alterations in AD are very complex and differ depending on age of onset of the disease and on the type of mutation, suggesting that different ganglioside-regulated events contribute to the onset of different AD forms. However, reduced ganglioside with altered ratios between a-series and b-series structures, and elevated levels of simpler gangliosides have been observed in different brain regions of AD and dementia of the Alzheimer type (DAT) affected patients (211-216) with respect to age-matched healthy controls. Consistent with these findings, downregulation of several glycosyltransferases was observed in dementia and AD patient brains (217). On the other hand, lipid rafts from the frontal and temporal cortices of AD patients contain a higher concentration of gangliosides GM1 and GM2 respect to age-matched control brains (218).

Anomalous organization of ganglioside-enriched membrane domains seems to be relevant to the functions, traffic and proteolytic cleavage of the main player in AD, the amyloid precursor protein (APP) (Figure 4). The exact physiological functions of APP are still to be fully elucidated (219), however it is apparently involved in the transduction across the membrane of signals relevant to neuronal adhesion, survival and synaptic functions

(220). Alterations in the membrane microenvironment of APP could alter the functions of APP as a signaling molecule (221). On the other hand, great emphasis in AD pathogenesis has been given to overproduction of Aβ peptides (that accumulate in the brain lesions that are commonly thought to cause AD (222)). Ganglioside-enriched lipid rafts contain not only APP, but also APP-derived proteolytic fragments, including Aβ, and the proteolytic enzymes involved in APP amyloidogenic processing. In particular they are enriched in active β and γ-secretases, and the production of Aβ amyloid is preferentially, even if not exclusively localized within lipid rafts (223). In other words, association with lipid rafts is a pre-requisite for the amyloidogenic processing of APP, which could be favored by altered lipid raft organization driven by changes in their ganglioside composition.This notion has been recently supported by the observation that the introduction of *B4galnt1*in cells lacking the expression of this enzyme (with concomitant increase in the cellular levels of GD2, GM2 and GM1), positively regulates APP β-cleavage (224). In turn, cleavage of APP also has a direct influence on the cellular lipid composition, since APP processing alters the synthesis of several lipids enriched in lipid rafts (225). Moreover, γ-cleavage of APP, also taking place in lipid rafts, leads to the release of the APP intracellular C-terminus domain (AICD), which has distinct physiological functions, regulating the expression of several genes (219), including serine-palmitoyltransferase (226), the key enzyme in ganglioside *de novo* biosynthesis. On the other hand, targeting of APP to lipid rafts is regulated via by the Src family kinase Fyn, associated with lipid rafts in a ganglioside-dependent way (227,228).

After the formation of soluble Aβ is formed by the amyloidogenic proteolytic cleavage the next step in the molecular mechanism underlying the onset of AD is Aβ conversion into aggregates, which are the seeds for the formation of insoluble amyloid fibrils. Soluble Aβ directly binds cholesterol and GM1 ganglioside (interacting with the ganglioside oligosaccharide chains by sugar-specific interactions) (229-232), and the binding of Aβ with

membrane GM1 (233,234), which is modulated by cholesterol, drives its conformational transition by favoring the conversion of the aggregated form (235) leading to Aβ fibrillogenesis (236) (Figure 4). GM1-driven Aβ aggregation requires the interaction with a GM1-rich membrane microdomain rather than a simple Aβ-GM1 interaction (237). On the other hand, interaction of GM1 with Aβ can lead to the formation of toxic soluble oligomers, that exert their detrimental effects through the high affinity binding with another protein enriched in lipid rafts, cellular PrP (238).

More recently, a role for GM3-dependent membrane organization has been proposed in relation to type 2 diabetes. In 3T3-L1 adipocytes, the induction of insulin resistance by prolonged treatment with low concentrations of TNFα (an experimental condition mimicking the chronic low-grade inflammation status typical of the obese adipose tissue) was accompanied by the upregulation of GM3 synthase, leading to an increase of cellular GM3 (239,240), that accumulated in detergent-resistant membranes. This alteration of membrane organization had profound consequences on the activity of insulin receptor in adipocytes (Figure 5). Insulin receptors (IR) are associated with detergent-resistant membranes from normal adipocytes (241) and localized in caveolae in intact cells (240). In caveolar membrane domains, the β-subunit of IR interacts with caveolin-1 through a binding motif recognizing the scaffold domain of caveolin-1 (242). On the other hand, IR can be engaged in a lateral interaction by GM3. The interaction between GM3 and IR is direct, since the receptor can be cross-linked to a photoactivable GM3 derivative, it is specific, since it is abolished by the addition of exogenous GM3 in co-immunoprecipitation experiments and involves the lysine residue at 944 in the aminoacid sequence of the receptor, suggesting that an electrostatic interaction between the negatively charged sialyllactose chain of GM3 and the positively charged amino group of lysine 944 is essential for the formation of the GM3/IR complex.Co-immunoprecipitation, cross-linking, fluorescence microscopy and

FRAP experiments showed that IR can form distinct complexes with caveolin-1 and GM3 within lipid membrane domains (243). Experimental increase of GM3 levels in normal adipocytes abolished insulin signaling and glucose uptake. Remarkably, in insulin-resistant adipocytes, the association of IR with GM3 was increased, while its association with caveolin-1 was decreased, indicating that the excess amount of GM3 in lipid membrane domains leads to the displacement of IR from the complex with caveolin-1. This suggests that the regulation of IR/caveolin-1 interaction by GM3 could be responsible for the changes in insulin response in adipocytes (243). This is a very nice example showing that the function of a plasma membrane-associated protein can be modulated by its ganglioside-driven dynamic partitioning between distinct membrane microdomains. Interestingly enough, the possible clinical relevance of this phenomenon has been recently highlighted by the finding that the increased serum levels of specific GM3 molecular species are associated with risk factors of metabolic syndrome (244).

CONCLUSIONS

The structural complexity of gangliosides oligosaccharide chains has attracted the attention of the researchers in this field for decades. Gangliosides head groups, protruding toward the extracellular space, are clearly designed by evolution to be engaged in interactions with molecules sitting on the same cellular membrane (*cis* interactions), or present in the extracellular environment or on the surface of other cells (*trans* interactions). Oligosaccharide-dependent recognition of gangliosides by interacting molecules is undoubtedly very important for the multifaceted biological functions of gangliosides. On the other hand, as discussed in this chapter, the chemico-physical properties of gangliosides do represent a major driving force for the organization of lateral order within biological membranes, and several biological functions of gangliosides are determined by this role, rather or in addition to the establishment of specific molecular interactions. Indeed, in some

cases ganglioside-driven clustering of membrane molecules seems to facilitate their interaction with gangliosides themselves at the membrane level. This leads to an interesting speculation. The possible significance of alterations in gangliosides levels associated with several pathological conditions might be linked to the consequent alterations in membrane organization. Targeting aberrant ganglioside-driven membrane organization, rather than simply targeting ganglioside metabolism, might represent a valuable therapeutic strategy for some diseases, as proposed by the group of Inokuchi for type 2 diabetes (245).

FIGURE LEGENDS

Fig. 1 Schematic representation of the volumes of phosphocholine and GM1 oligosaccharide, based on the possibleminimum energy conformations estimated by MM2 force field analysis.

Fig. 2 Schematic representation of phase separation driven by the differences in the oligosaccharide chains in a GM2/GT1b micelle (reproduced from (27)).

Fig. 3 Remodeling of the plasma membrane ganglioside pattern by the local activity of hydrolytic enzymes or glycosyltransferases. Panel A: Changes of ganglioside glycosylation can affect either the clustering of signaling proteins within ganglioside-enriched membrane domains, or the membrane curvature. Panel B: schematic representation of the stepwise removal of saccharide units from the ganglioside oligosaccharide head group by the action of different hydrolytic enzymes, whose activity has been reported at the plasma membrane level.

Fig. 4 Targeting of APP to lipid raft with anomalous lipid composition can direct APP toward its amyloidogenic proteolytic processing, leading to the generation of amyloid Aβ peptide. Membrane bound Aβ can interact with ganglioside-rich domains at the cell surface with several different possible consequences: the trigger of the formation of insoluble amyloid fibrils, or the release of toxic soluble Aβ aggregates. In addition, association with anomalous

lipid rafts can affect APP-mediated signal transduction. sAPPβ, soluble form of APPβ, released by β-cleavage of APP. AICD, APP intracellular C-terminus domain, released by subsequent γ-cleavage of APP. Aβ, amyloid Aβ peptide, generated by amyloidogenic processing of APP. Reproduced from (246) with permission.

Fig. 5 Schematic representation of the mechanism behind the shift of IR from the caveolae to glycolipid-enriched membrane domains (GEM) in insulin-resistant adipocytes. Binding of IR and caveolin-1 in caveolar domains is required for effective signal transduction downstream to IR. In insulin-resistant adipocytes, IR is sequestered in GM3-rich membrane domains and thus displaced from its association with caveolin-1. Reproduced from (243) with permission. Copyright (2007) National Academy of Sciences, U.S.A.

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