Biochemistry

 Volume 29, Number 49 December 11, 1990

Perspectives in Biochemistry

Metabolism and Intracellular Transport of Glycosphingolipids†

Günter Schwarzmann* and Konrad Sandhoff*

Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Strasse 1, D-5300 Bonn 1, FRG

Received April 26, 1990; Revised Manuscript Received July 10, 1990

Apart from the phospholipids that constitute the majority of building blocks of biological membranes, glycosphingolipids are characteristic components of eukaryotic plasma membranes and as such are ubiquitous in vertebrate tissues (Wiegandt, 1982, 1985). A special family of acidic glycosphingolipids comprises the gangliosides (Figure 1) that are present in especially high concentrations in neuronal plasma membranes (Ledeen, 1985; Hansson et al., 1977). Glycosphingolipids of the plasma membrane are generally known to be anchored in the outer leaflet of the membrane bilayer by their hydrophobic ceramide moiety (Gahmberg & Hakomori, 1973; Thompson & Tillack, 1985). The hydrophilic oligosaccharide residues of neutral glycosphingolipids and gangliosides protrude into the extracellular space and partially cover the cell surface. Together with membrane glycoproteins and proteoglycans, these carbohydrate residues constitute the glycocalyx of the cell surface. Besides their location in plasma membranes, some specific glycosphingolipids may also be highly concentrated at intracellular membranes, e.g., lactosylceramide at cytoplasmic granular membranes in neutrophils (Symington et al., 1987).

Glycosphingolipids, whose biological roles are gradually emerging, are thought to play a role in cell differentiation and morphogenesis (Hakomori, 1984a) and may conceivably contribute to the protection of the cell against environmental fluctuations. Though gangliosides have been identified as specific binding sites for viruses and toxins (Svennerholm, 1985) and have been implicated in the modulation of membrane-bound receptors (Bremer et al., 1986; Hanai et al., 1988) and in the control of the cell cycle (Usuki et al., 1988), the precise nature of their functional role and their seeming influence on overall membrane dynamics have yet to be defined.

On the cell surface glycosphingolipids, including gangliosides, form cell-specific patterns which are thought to be important for cell adhesion and which change specifically with cell differentiation, morphogenesis, and oncogenic transformation (Hakomori, 1981; Fenderson et al., 1985)). During ontogenesis of the murine nervous system, the ganglioside content increases drastically and the glycosphingolipid pattern on the neuronal cell surface changes (Seyfried et al., 1984).

Although the plasma membrane is surmised to be the sole place for the functional action of glycosphingolipids, it is, however, neither the site of glycosphingolipid biosynthesis nor of their degradation. Thus, the maintenance of a balanced glycosphingolipid profile demands a stringent control of glycosphingolipid metabolism and glycosphingolipid intracellular transport.

Biosynthesis and Transport

During the life of a cell, extensive membrane flow by endo- and exocytic processes takes place, and specific sorting and other regulatory mechanisms must be operative to safeguard the identity of individual glycosphingolipid patterns in plasma membranes.

Much of our current knowledge on glycosphingolipid traffic in cells has been derived from metabolic studies. Glycosphingolipid biosynthesis, like glycoprotein biosynthesis, is coupled to movement along a membrane pathway from the endoplasmatic reticulum (ER) to the Golgi compartments, eventually delivering glycosphingolipids to the plasma membrane (Figure 2). While biosynthesis of N-linked glycoproteins and their trafficking mechanisms have been well-established, the site of biosynthesis of glycosphingolipids and their transport mechanisms are still not well-defined. Although clear experiments that would indicate the site of ceramide biosynthesis are still lacking, the lipophilic backbone of glycosphingolipids is assumed to be synthesized in the ER. The exact location of the nex step in the synthesis of gangliosides and the major neutral glycosphingolipids, i.e., glucosylation of ceramide, is not yet clear but could be either in the ER (Suzuki et al., 1984) or on the cytosolic face of the Golgi (Coste et al., 1985, 1986) or in the cis Golgi/ER transitional elements. Addition of subsequent sugars to glucosylceramide to give trihexosylceramides, thus defining the core structure and diversity of the glycosphingolipid species (ganglio, globo, lacto, and muco structures), presumably takes place in the

† The work of G.S. and K.S. referred to in this paper was financially supported by the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie.

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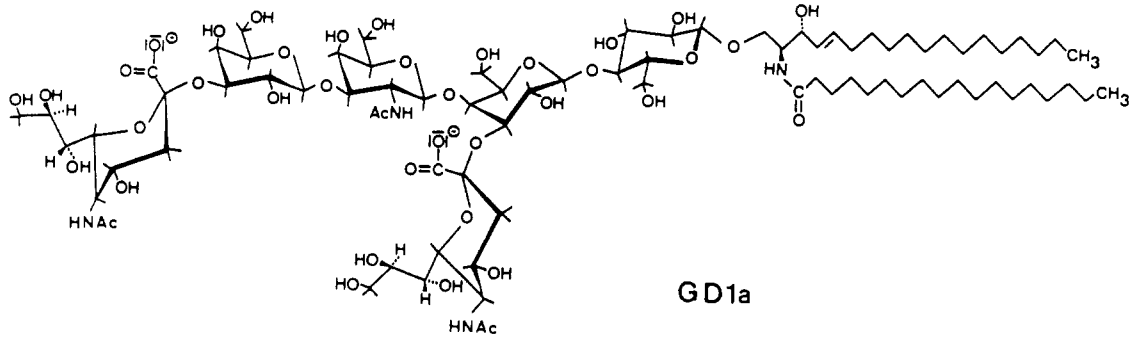

FIGURE 1: Structure of ganglioside GD1a.

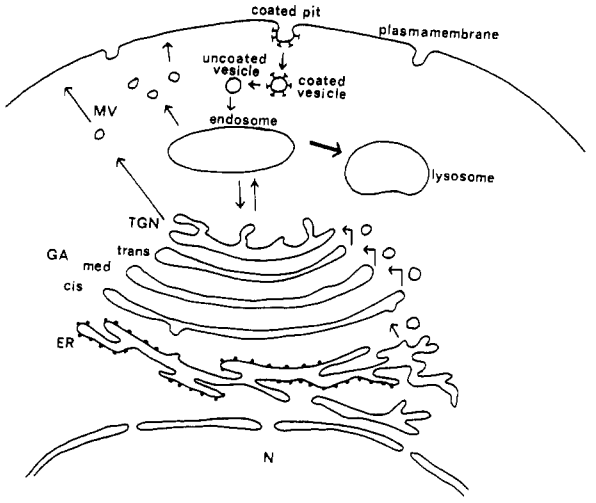

FIGURE 2: Major compartments of glycosphingolipid metabolism. Biosynthesis of glycosphingolipids is catalyzed by membrane-bound transferases of the endoplasmic reticulum (ER) and cisternae of the Golgi apparatus (GA) and trans Golgi network (TGN). Glycolipids of the plasma membrane can reach the lysosomal compartment by a vesicular endocytotic pathway; their catabolism is facilitated by exohydrolases and glycolipid binding proteins (activators) in the lysosomes. MV, membrane vesicles; N, nucleus.

Golgi complex. The glycosyltransferases involved in the sequential transfer of monosaccharide or sialic acid residues from their appropriate sugar nucleotides to the growing oligosaccharide chain have been shown to be membrane-bound and restricted to the lumen of the Golgi cisternae (Carey & Hirschberg, 1981; Creek & Morrê, 1981; Fleischer, 1981; Yusuf et al., 1983a,b).

Exactly where the individual glycosylation reactions take place in the Golgi stack still awaits a definite answer. However, initial clues arise from competition experiments demonstrating that in Golgi vesicles, derived from rat liver, the addition of *N*-acetylgalactosamine and galactose to the growing gangliosides is catalyzed by one respective enzyme; i.e., GalNAc transferase is responsible for GM2 and GD2 and Gal transferase II for GM1 and GD1b synthesis, respectively (Figure 3). Likewise, one single sialyltransferase IV is capable of synthesizing GD1a and GT1b (Pohlentz et al., 1988; Iber et al., 1989), and one single sialyltransferase V seems to be responsible for the synthesis of GT1a and GQ1b (Iber & Sandhoff, 1989). The sialyltransferase IV was shown to be different from sialyltransferase II and possibly also from sialyltransferase I, which catalyze formation of GD3 and GM3, respectively (Pohlentz et al., 1988). Enzyme kinetic experiments with rat liver derived Golgi vesicles indicate, however, that it is likely that sialyltransferase I can catalyze formation of both GM3 and GD1a from LacCer and GM1, respectively (unpublished). From these experiments it is, however, not clear whether sialyltransferases I and IV are one and the same

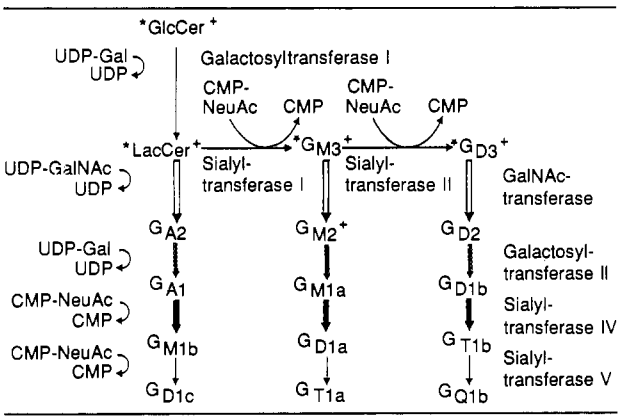

FIGURE 3: General scheme for ganglioside biosynthesis (Pohlentz et al., 1988). Asterisks indicate glycosphingolipids accumulating in the presence of brefeldin A. Pluses indicate glycosphingolipids accumulating in the presence of monensin (van Echten et al., 1990a).

enzyme or isoenzymes at more than one site of the Golgi stack. Nevertheless, sialyltransferase I has been localized recently in the cis Golgi compartment and sialyltransferase IV in the trans Golgi compartment of rat liver (Trinchera & Ghidoni, 1989). These results strongly suggest that ganglioside biosynthesis occurs along movement through different cisternae of the Golgi with the formation of less and higher glycosylated gangliosides in cis- and trans-oriented compartments of the Golgi stack, respectively.

Drugs Affecting Glycosphingolipid Biosynthesis

Additional evidence for different sites of ganglioside biosynthesis in the Golgi apparatus comes from studies on drug-induced Golgi dissection. As was shown for cultured murine primary neurons, the cationic ionophore monensin has a pronounced effect on ganglioside biosynthesis. In the presence of this drug incorporation of radioactivity from [^{14}C]galactose decreases remarkably for the gangliosides GM1, GD1a, GD1b, GT1b, and GQ1b, whereas relative labeling of GlcCer, LacCer, GM3, GD3, and GM2 is increased significantly, with the radiolabel appearing most prominently in GlcCer (van Echten & Sandhoff, 1989). Since monensin has been shown to impede vesicular membrane flow, primarily acting between the proximal and distal regions of the Golgi apparatus [reviewed by Tartakoff (1983)], the results (see above) suggest that complex gangliosides, for example, GT1a and GQ1b, are synthesized in the trans-oriented Golgi cisternae and the less glycosylated gangliosides such as GM3 and GD3 as well as GlcCer and LacCer in cis-oriented Golgi elements. Similar effects of monensin on the incorporation of radioactivity from galactose into glycosphingolipids were reported previously. Thus, in cultured neurotumor cells a profound increase in labeled GlcCer and a reduction in labeled gangliosides as well as in $GbOse_3Cer$ and $GbOse_4Cer$ were observed

(Miller-Prodraza & Fishman, 1984). This monensin-induced increase in radiolabel incorporation into GlcCer and LacCer with a concomitant reduction of label incorporation into complex gangliosides, as well as $GbOse_3Cer$ and $GbOse_4Cer$, has also been reported for cultured human fibroblasts (Saito et al., 1984, 1985). These results again argue for the synthesis of the more highly glycosylated neutral glycosphingolipids and gangliosides in the Golgi complex at a site or sites that are beyond the transport block elicited by monensin.

Further evidence for the biosynthesis of complex gangliosides in the trans stacks of the Golgi apparatus stems from studies on the influence of brefeldin A (BFA) on the incorporation of labeled precursors into neutral glycosphingolipids and gangliosides (van Echten et al., 1990a). Brefeldin A (Härri et al., 1963) has been reported to block vesicular transport from the ER to the cis Golgi (Takatsuki & Tamura, 1985; Misumi et al., 1986) and to cause vesiculation of the Golgi, whereby the former medial and cis compartments fuse with the ER (Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989; Doms et al., 1989). The trans Golgi may also possibly be involved to some extent in this fusion process (Lippincott-Schwartz et al., 1990).

Whatever labeled precursors (galactose, serine, sphingosine, or palmitic acid) were used, under the influence of BFA a dramatic reduction of label incorporation into gangliosides GM1, GD1a, GT1b, and GQ1b of primary cultured murine cerebellar cells with concurrent label accumulation in GlcCer, LacCer, GM3, and GD3 was observed (van Echten et al., 1990a). These results indicate that the latter glycosphingolipids are synthesized in the cis Golgi or closely neighboring compartments whereas complex gangliosides such as GM1, GD1a, GT1b, and GQ1b are synthesized beyond the drug-induced transport block. So far no clear indication as to the location of GM2 synthase can be gained from these observations. If GM2 is synthesized before the trans Golgi compartment, as implied by van Echten and Sandhoff (1989), then an unaltered or even an increased label uptake by this ganglioside should occur in the presence of BFA; this was not observed. It is obvious and perhaps likely that BFA, like other drugs impeding membrane transport, may affect multiple cellular processes, and as long as the effects of these drugs on the Golgi apparatus and the ER are not completely understood, an unambiguous interpretation of these results, with the aim of localizing most of the glycosyltransferases at distinct sites in these organelles, is at present not possible. Thus, further studies on subcompartmentation of the Golgi are needed to delineate accurately the sites at which glycosylation of the growing oligosaccharide chains takes place.

Despite the fact that the biosynthesis of gangliosides and neutral glycosphingolipids takes place in at least two different sites, and that the specificities of some glycosyltransferases have been well characterized (see above), the regulation of the biosynthesis of these lipids is still poorly understood.

Regulation of Biosynthesis

Ganglioside GM3 is the mutual precursor for both GM2 and GD3 synthesis, constituting the branching point where a- and b-series gangliosides diverge. Thus, GM2 and GD3 synthases may well have key regulatory roles. Their activities may depend strongly, more so than those of the other glycosyltransferases, on environmental factors such as adjacent lipids, divalent cations, and availability of sugar nucleotides (Sommers & Hirschberg, 1982; Yusuf et al., 1983a,b). Quite recently it was shown that changing the pH from 7.4 to 6.2 in the culture medium of murine cerebellar cells resulted in a reversible shift from a- to b-series in ganglioside biosynthesis. This can be explained by the different pH profiles of the transferases involved. At pH 6.2 sialyltransferase II, a key enzyme in the biosynthesis of b-series gangliosides, is more active than GalNAc transferase, the first enzyme in the synthesis of a-series gangliosides; however, at pH 7.4 the opposite is true (Iber et al., 1990).

Moreover, rates of glycosphingolipid biosynthesis in general probably depend on the accessibility of sphingosine for ceramide formation (van Echten et al., 1990b). When primary cultured murine cerebellar cells were fed with natural sphingosine or its homologues with either 12 or 24 carbon atoms or with biosynthetically inactive 2-azido-1,3-dioxyoctadecane ("azidosphingoid"), an inhibition of endogenous sphingosine synthesis was observed, suggesting a control of sphingosine biosynthesis by endogenous sphingosine levels.

A feedback control of ganglioside biosynthesis is suggested by in vitro experiments (Nores & Caputto, 1984; Yusuf et al., 1987). In these experiments GM2 synthase was the most strongly inhibited by GD1a and GD3 synthase by GQ1b. These results indicate that the regulatory steps in the synthesis of a- and b-series gangliosides are preferentially inhibited by their respective end products. However, this kind of mechanism has not yet been observed in cell culture or in vivo.

In addition to epigenetic regulation, ganglioside biosynthesis can be genetically controlled at the transcriptional level of the respective glycosyltransferases (Hashimoto et al., 1983; Nakakuma et al., 1984; Nagai et al., 1986). There is abundant evidence that terminal glycosylation sequences in glycoproteins and complex glycosphingolipids are differentially expressed in cells and are subject to change during development, differentiation, and oncogenic transformation [reviewed by Hakomori (1984b), Feizi (1985), and Rademacher et al. (1988)]. Several reports have demonstrated that the changes in glycolipid or glycoprotein glycosylation in transformed cells correspond to quantitative or qualitative changes in the expression of the relevant glycosyltransferases (Coleman et al., 1975; Nakaishi et al., 1988a,b; Matsuura et al., 1989).

Pathways of Intracellular Transport

In general, two major pathways of intracellular glycosphingolipid flow in vertebrate cells can be discriminated. First, there is a translocation of newly synthesized glycosphingolipids from the Golgi complex to the plasma membrane, and second, there is a glycosphingolipid flow in the opposite direction from the plasma membrane to intracellular organelles during endocytosis. A number of intermediate compartments, the endosomes during inbound and the Golgi complex during outbound traffic, play crucial roles in defining the destinations for the glycospingolipids. Both pathways may act by default, and unless specific sorting occurs, glycosphingolipids within the membrane structures associated with both routes will end up in lysosomes or the plasma membrane following endo- and exocytosis, respectively.

In neurons, which are highly enriched in plasma membrane bound gangliosides and with a tremendous network of processes, the intracellular ganglioside translocation is more complex than in nonneuronal cells. In the central nervous system gangliosides seem to be synthesized predominantly in the cell body of nerve cells [reviewed by Ledeen (1989)]. From their site of synthesis gangliosides are conveyed to axonal and nerve-ending membranes by fast axonal transport, as has been shown in the optic system of fish (Forman & Ledeen, 1972; Rösner et al., 1973), chick (Rösner, 1975; Landa et al., 1979), rabbit (Ledeen et al., 1981), and rat (Gammon et al., 1985). In these studies radiolabeled ganglioside precursors were used. Fast axonal transport of exogenously applied ganglioside has

also been demonstrated in the optic system of chick (Willibald et al., 1988). Following injection into one eye, authentic [^{3}H]GD1a, besides some partial degradation, progressed rapidly in an anterograde direction throughout the entire optic system (optic nerve, optic chiasm) within 1 day.

Exocytosis

Since glycosphingolipid synthesis is accomplished on the lumenal side of subsequent Golgi cisternae, and as glycosphingolipids have been shown not to undergo transbilayer diffusion (flip-flop), it is definitely apparent that they must be translocated to the plasma membrane by vesicular membrane flow.

A vesicular membrane flow of a fluorescently tagged analogue of GlcCer (NBD-GlcCer) and of sphingomyelin (NBD-SM), in which the fatty acyl moiety was replaced by a *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-6-aminocaproyl residue, through the Golgi apparatus to the plasma membrane has been demonstrated elegantly in cultured Chinese hamster lung fibroblasts (Lipsky & Pagano, 1985a). These fluorescent spingolipid analogues were synthesized intracellularly from exogenously supplied NBD-ceramide and were translocated to the plasma membrane with a half-time for transport of about 20–30 min at 37 °C (Lipsky & Pagano, 1983, 1985a). This is consistent with the value reported for the appearance of a newly synthesized radiolabeled ganglioside at the plasma membrane (Miller-Podraza & Fishman, 1982). Furthermore, intracellular transport of NBD-GlcCer and -SM was shown to be inhibited in mitotic cells (Kobayashi & Pagano, 1989). These findings strongly suggest that these fluorescent sphingolipids, and most likely all natural sphingolipids after synthesis from endogenous ceramide, progress through the Golgi apparatus to the cell surface via vesicular membrane flow.

Whether glycosphingolipid transport along the biosynthetic pathway is totally unidirectional or allows for retrograde movement from distal to proximal regions of the Golgi apparatus is at present not definitely clear. From studies with an endogenously synthesized NBD-GlcCer, it appears that no transport of glycosphingolipids into the ER occurs (Simons & van Meer, 1988). In contrast, vesicular transport at least from the salvage compartment (post ER compartment) back to the ER has been demonstrated for some ER resident proteins (Pelham, 1988, 1989). This finding implies that glycosphinoglipids are conveyed through the Golgi cisternae by a default pathway similar to, if not identical with, the biosynthetic protein transport in fibroblasts (Wieland et al., 1987) without sorting in their early transport pathway. Indeed, as was shown recently by comparing glycosphingolipid transport with transport of the membrane glycoprotein ("G" protein) of vesicular stomatitis virus (Wattenberg, 1989), both processes seem to be indistinguishable kinetically. Moreover, both processes have requirements for nucleotide triphosphates, intact membranes, a high molecular weight cytosolic fraction, and an *N*-ethylmaleimide-sensitive factor. If glycospingolipids and glycoproteins are indeed cotransported, there is no compelling reason to suspect retrograde transport in the Golgi stack as well as to postulate a positive transport signal for any glycolipid. However, sorting of a fluorescent analogue of GlcCer prior to delivery to the apical plasma membrane has been shown to take place most reasonably in the trans Golgi network of polarized (MDCK) cells (Simons & van Meer, 1988; van Meer, 1989), and this mechanism may also hold for most, if not all, natural glycosphingolipids in simple epithelia. It has been speculated that the mechanism by which this sorting occurs may be signal-mediated and may involve clustering of glycosphingolipids in an apical precursor microdomain in the lumenal leaflet of the trans Golgi network (Simons & van Meer, 1988).

Endocytosis

Studies of turnover rates for gangliosides in rat brain have given highly variable results, owing in part to inherent differences among systems as well as negligence to correct for precursor reutilization. Thus, for gangliosides, half-lives varying from a few days to several weeks have been reported [reviewed by Ledeen (1989)]. Considering the fact that cultured cells can internalize approximately half their surface area per hour (Steinman et al., 1983), these rather lengthy half-lives imply that either most of the surface-bound glycosphingolipid molecules do not participate in every endocytic event or that they recycle many times between the endosomes and the plasma membrane prior to being finally delivered to lysosomes.

Previously, it has been shown in Chinese hamster ovary cells that NBD-sphingomyelin internalized from the plasma membrane recycles between intracellular compartments and the plasma membrane (Koval & Pagano, 1989). As both fluorescent sphingomyelin and transferrin, a protein known to be recycled, were transported through similar intracellular compartments in these cells, the authors concluded that endocytosed NBD-sphingomyelin is transported to sorting endosomes or CURL (compartment of uncoupling of receptor and ligand; Geuze et al., 1983, 1987). Most of the fluorescent sphingomyelin has been found to return to the plasma membrane from these "early" endosomes with some being delivered to the lysosomes. Comparable results were obtained with NBD-GlcCer in baby hamster kidney cells (Kok et al., 1989). The fluorescent GlcCer analogue was found to comigrate with transferrin to the early endosomes in these cells and to be able to recycle to the plasma membrane.

An important question is whether natural glycosphingolipids can also undergo recycling along a receptor-mediated pathway. A further question is whether gangliosides and neutral glycosphingolipids are subject to sorting within the plasma membrane by specific interactions with, for example, cell surface proteins. Ganglioside binding proteins have been inferred from studies with cultured human fibroblasts (Sonnino et al., 1989) and rat brain membranes (Tiemeyer et al., 1989), suggesting that endocytosis of glycolipids might also be controlled by membrane composition.

In order to explore endocytosis of gangliosides in more detail, studies with labeled exogenous gangliosides have been performed. This approach allows one to study the fate of a single labeled ganglioside; this is not possible with metabolically labeled endogenous gangliosides. These labeled exogenous gangliosides can insert into the plasma membrane of cultured cells and mix with the pool of endogenous glycosphingolipids, as has been demonstrated by electron spin resonance spectroscopy (Schwarzmann et al., 1983, 1986).

Thus far, there is some experimental evidence that during inbound traffic gangliosides are internalized via coated pits. Few ganglioside molecules escape transport into lysosomes and are diverted to the Golgi complex. This has been shown by electron microscopic studies employing biotin–GM1, carrying a biotinyl rather than an acetyl group in its sialic acid moiety, and indirectly by metabolic studies of radiolabeled gangliosides and some of their derivatives. By virtue of the biotin residue this ganglioside derivative can bind gold/streptavidin and thus be visualized by electron microscopy. Binding of gold/streptavidin to ultrathin slices of fibroblasts derived from a patient suffering from Tay-Sachs disease, that had been pretreated with biotin–GM1, revealed that the biotin label was

confined largely to coated pits, to endocytotic vesicles, to lysosomes, and to the membranes of the Golgi complex (Schwarzmann et al., 1987). This experiment clearly demonstrates that exogenous gangliosides, once inserted into the outer leaflet of the plasma membrane of cultured cells, are subject to endocytosis via coated pits and, besides being transported to lysosomes, are also delivered to the Golgi apparatus.

The amount of ganglioside transported to the Golgi complex is, however, rather small compared with that ending up in lysosomes. This is evidenced by experiments using the fluorescently tagged gangliosides NBD-GM3, -GM2, -GM1 and -GD1a. These semisynthetic derivatives (Schwarzmann & Sandhoff, 1987), by virtue of their short-chain fatty acids, rapidly and spontaneously transfer to biological membranes and, thus, can be monitored easily for their intracellular distribution by fluorescence microscopy, as has been demonstrated previously for NBD-ceramide (Lipsky & Pagano, 1983).

When applied to cultured human fibroblasts, under conditions of endocytotic block, these fluorescent ganglioside derivatives as well as NBD-LacCer and -GlcCer specifically insert into the outer leaflet of the plasma membrane. On resumption of endocytosis, the labeled glycolipids are internalized and metabolized according to known pathways. As opposed to NBD-gangliosides and NBD-LacCer that predominantly label lysosomes, NBD-GlcCer gives rise mainly to Golgi labeling (unpublished). We could demonstrate that this Golgi labeling is caused by deglucosylation of NBD-GlcCer to NBD-ceramide, which then preferentially labels the Golgi apparatus (Lipsky & Pagano, 1985b), rather than by a specific transport mechanism in these cells; this was shown by using a nondegradable NBD-glucosylthioceramide, which accumulated in lysosomes with no detectable Golgi labeling. Furthermore, in human fibroblasts no products of glycosylation could be detected either, although this glucosylthioceramide readily accepted galactose from UDP-Gal in a LacCer synthase assay with rat liver derived Golgi vesicles (unpublished). Thus, it seems that in endocytosis the bulk of glycolipids that are diverted from recycling are targeted to lysosomes. However, a small percentage of endocytosed gangliosides is obviously also diverted to the Golgi apparatus as indirectly demonstrated by metabolic studies.

Direct Glycosylation of Plasma Membrane Derived Gangliosides

In various types of cultured human fibroblasts deficient in GM2 catabolism, labeled GD1a was formed by successive glycosylation of the administered intact labeled GM2 (Sonderfeld et al., 1985). Direct glycosylation of precursor gangliosides to yield GD1a has also been observed in rat liver after intravenous injection of labeled GM1, irrespective of its label position (Ghidoni et al., 1986). Moreover, when fibroblasts were fed with 10^{-5} M tritium-labeled GM2-amide for 48 h at 37 °C, the analysis of the lipid extract of these cells showed the formation of GD1a-amide (Klein et al., 1987a). Previously, it had been shown that GM2-amide cannot be degraded beyond GM3-amide, as this derivative is resistant to the action of sialidase (Klein et al., 1987b). Since GD1a-amide is not produced in cells fed with GM3-amide, it can be concluded safely that GD1a-amide is derived from GM2-amide by direct glycosylation (Klein et al., 1987a). However, when GM3 was incorporated in ceramidase-deficient fibroblasts from a patient suffering from Farber's disease, no products of glycosylation could be detected (Schwarzmann et al., 1986). Thus, it is evident that in normal cells fed with GM3 the glycosylation product, i.e., GD1a, must have resulted from de novo synthesis with incorporation of tritium-labeled sphinganine derived from complete degradation of exogenous GM3.

Results obtained with ganglioside-amides support these findings (Klein et al., 1987a). Subcellular fractionation studies on fibroblasts fed with GM3-amide revealed that most of this derivative was internalized and directed to the lysosomes, probably by a vesicular membrane flow. The lack of GD1a-amide formation from GM3-amide, in contrast to GM2-amide, suggests that either no transport into the Golgi complex of GM3-amide took place or that transport occurred into those cisternae that are downstream of GM2 and GD1a synthesis. In other words, GM3-amide as well as GM3 movement into the Golgi apparatus may be restricted to the trans Golgi compartment rather than to the cis Golgi compartment. In support of this view is the finding (Stotz, 1990) that in cultured human fibroblasts deficient in GM1 degradation (GM1 gangliosidosis) a small amount of internalized NBD-GM1 is glycosylated to NBD-GD1a even in the presence of BFA, a drug known to interrupt membrane flow from cis to trans Golgi cisternae (see above). However, when GlcCer, labeled in its glucose moiety, was injected into rats, labeled gangliosides that carried the label exclusively in the glucose residue could be detected in the liver (Trinchera et al., 1990). These authors inferred from their results that authentic GlcCer rather than the labeled glucose residue was incorporated into labeled gangliosides without giving any indication as to the site(s) of biosynthesis at which the intact labeled GlcCer entered.

Can Intact Glycosphingolipids Escape from Lysosomes?

A proportion of gangliosides that are translocated by endocytosis to the Golgi compartment obviously bypass the lysosomes. The possibility that gangliosides can escape from lysosomes and then reach the Golgi apparatus seems highly unlikely for the following reasons: When hexosaminidase-deficient fibroblasts of a patient suffering from Tay-Sachs disease were fed with GM1 tritium-labeled in its terminal galactose residue and radiocarbon-labeled in its ceramide portion, doubly labeled GD1a was formed (Klein et al., 1988). There was no significant change in the ratio of tritium to radiocarbon, indicating that no catabolic product of ganglioside GM1 (e.g., GM2, GM3, LacCer, GlcCer, or ceramide) could have left the lysosomes and been glycosylated in the Golgi complex. Similar results were obtained when normal fibroblasts were fed simultaneously with GM1-amide, tritium-labeled in its terminal galactose residue, and with [^{14}C]galactose. However, formation of GD1a-amide without any incorporation of [^{14}C]galactose indicated again that the GD1a-amide had originated exclusively from GM1-amide and not from the GM2-amide formed in the lysosomes from incorporated GM1-amide. As shown above, however, GD1a and GD1a-amide are also produced from exogenous GM2 and GM2-amide inserted into the plasma membrane rather than originating from lysosomes.

On the other hand, it has been claimed, from studies in living rats, that labeled intact ceramide and GlcCer, derived from the catabolism of intravenously injected labeled GM1, give rise to labeled gangliosides in the liver (Ghidoni et al., 1986; Tettamanti et al., 1988). If these labeled catabolites were to leave lysosomes of some liver cells and to enter the biosynthetic pathway (this was not shown by these authors), then their observations would be contrary to our findings (see above), demonstrating that (i) a nondegradable NBD-GlcCer accumulates in lysosomes of cultured fibroblasts and does not yield to glycosylation and that (ii) labeled ceramide derived from

catabolism of labeled GM3 accumulates in ceramidase-deficient fibroblasts (Farber disease) but does not enter the biosynthetic pathway.

From these metabolic studies of exogenous gangliosides and their amides, it is quite evident that once glycosphingolipids are inserted into the plasma membrane of cultured cells, they are then internalized and ultimately metabolized with the formation of a small percentage of higher glycosylated compounds. Further investigations are still necessary to unambiguously establish the intracellular traffic routes of glycosphingolipids and the exact sites of their biosynthesis. This aspect is important in view of the regulation of glycosphingolipid biosynthesis through the effects of their concentration and composition on the cell surface.

ACKNOWLEDGMENTS

We thank Dr. R. A. Klein for reading the manuscript.

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