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Review

Distinct roles for sphingolipids and glycosphingolipids at different stages of neuronal development*°

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Studies on the roles of sphingolipids (SLs) and glycosphingolipids (GSLs) at distinct stages of neuronal development have been performed using primary cultures of hippocampal neurons, which are unique among neuronal cultures inasmuch as they develop by a well-characterized and stereotypic sequence of events that gives rise to fully differentiated axons and dendrites. Our data demonstrate that SLs and GSLs play at least three distinct roles in regulating neuronal development, namely: (i) ceramide enhances the formation of minor neuronal processes from lamellipodia and the subsequent stage of axonogenesis; (ii) glucosylceramide synthesis, but not the synthesis of higher-order GSLs, is required for normal axon growth and for accelerated axonal growth upon stimulation by growth factors; and (iii) at both of these stages, ceramide at high concentrations can induce apoptotic cell death. Together, these observations are consistent with the possibility that minor process formation and apoptosis are regulated by ceramide-dependent signaling pathways, whereas axonal growth requires glucosylceramide synthesis, perhaps to support an intracellular transport pathway.

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Abbreviations: BDNF, brain-derived neurotrophic factor; CBE, conduritol B-epoxide; bFGF, basic fibroblast growth factor; GlcCer, glucosylceramide, GSL, glycosphingolipid; N-SMase, neutral-sphingomyelinase; C_6-NBD-ceramide, N-[6-[(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)amino]hexanoyl-D-erythro-sphingosine; NGF, nerve growth factor; NTR, neutrophin; D-PDMP, D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; SL, sphingolipid.

Over the past few years, our laboratory has initiated a series of studies to examine the roles of sphingolipids (SLs) and glycosphingolipids (GSLs) in neuronal growth and development. Although it has been known for many years that neurons are enriched in GSLs, particularly the gangliosides (sialic-acid containing GSL), the precise function(s) of these intriguing molecules has not been clearly defined. This is in part due to the large variety of experimental systems used and to the large number of GSLs that have been studied. Conclusions drawn about the function of a particular GSL in one neuronal culture system may not be applicable to other systems, or to another GSL.

In order to ascribe precise roles to specific SLs and GSLs at defined stages of neuronal development, we have adopted two novel experimental approaches. First, we used well-characterized primary cultures of hippocampal neurons, rather than the neuronal cell lines (e.g. neuroblastoma cells) that have been used in the majority of studies. Secondly, the use of inhibitors of SL metabolism permitted examination of the role of *endogenous* SLs and GSLs, rather than exogenously added SLs, in neuronal growth. The latter point is of particular importance as it alleviates the need to determine whether the exogenously added GSLs are fully integrated into biological membranes, and whether effects subsequently observed actually mimic a physiological function of the GSL rather than a pharmacological, 'non-specific' effect.

The use of a defined culture system, namely hippocampal neurons, also merits some discussion. Hippocampal neurons cultured according to protocols developed by Banker and colleagues over the past 20 years (Goslin & Banker, 1991) are almost unique in neuronal cultures inasmuch as they develop by a stereotypic sequence of events giving rise to fully differentiated neurons, in which axons and dendrites can be distinguished both biochemically and anatomically. Their growth has been well-characterized and classified according to five distinct developmental stages (Dotti *et al.*, 1988).

In the initial stage of growth (stage 1), hippocampal neurons are characterized by the presence of many lamellipodia around the cell body. The second stage of development is marked by loss of lamellipodia and extension of a number of short processes, designated 'minor processes' (stage 2). After some hours, one of the minor processes starts to grow rapidly (10–15 μm/h) and develops axonal characteristics (stage 3). Axons form branches as collaterals, and as each new branch emerges, the growth cone of the original axon loses its lamellipodial appearance and elongation stops. Dendrites develop from minor processes (stage 4), and the final stage of development (stage 5) is characterized by the formation of synaptic contacts between axons and dendrites.

Using this culture system we have defined the role of particular SLs and GSLs in the growth of both axons and dendrites, and have analyzed their roles in the development of neuronal polarity. Our major findings are summarized in Fig. 1, and will be discussed briefly in the first part of this review. In the second part, evidence will be discussed to demonstrate that ceramide and glucosylceramide (GlcCer), the simplest GSL, play regulatory roles at different stages of neuronal development.

GANGLIOSIDES SYNTHESIS, CONTENT, LOCALIZATION AND ENDOCYTOSIS DURING NEURONAL DEVELOPMENT

Over the past 4–5 years, our laboratory has published a number of studies describing the synthesis, content, rate of endocytosis and localization of gangliosides during neuronal development, and, in particular, during the development of neuronal polarity. In these studies we focused on gangliosides, rather than on the neutral or other GSLs, since gangliosides

have been postulated to play a variety of important functions in neurons, including roles in neuritogenesis, receptor function and synaptic transmission (Ledeen & Yu, 1992).

The first unexpected result was obtained when we analyzed the synthesis of gangliosides at defined stages of development (see Fig. 1). The major changes in ganglioside synthesis occurred mainly in the period of axonogenesis and rapid axon growth (Hirschberg *et al.*, 1996); during axonogenesis, there was a significant increase in the synthesis of complex gangliosides (i.e. G_{M1}, G_{D1a}, G_{D1b} and G_{T1b}) with a corresponding reduction in the synthesis of glucosylceramide and ganglioside G_{D3}. During the stage of rapid axon growth, the ratio of a- to b-series gangliosides increased significantly. In contrast, during den-

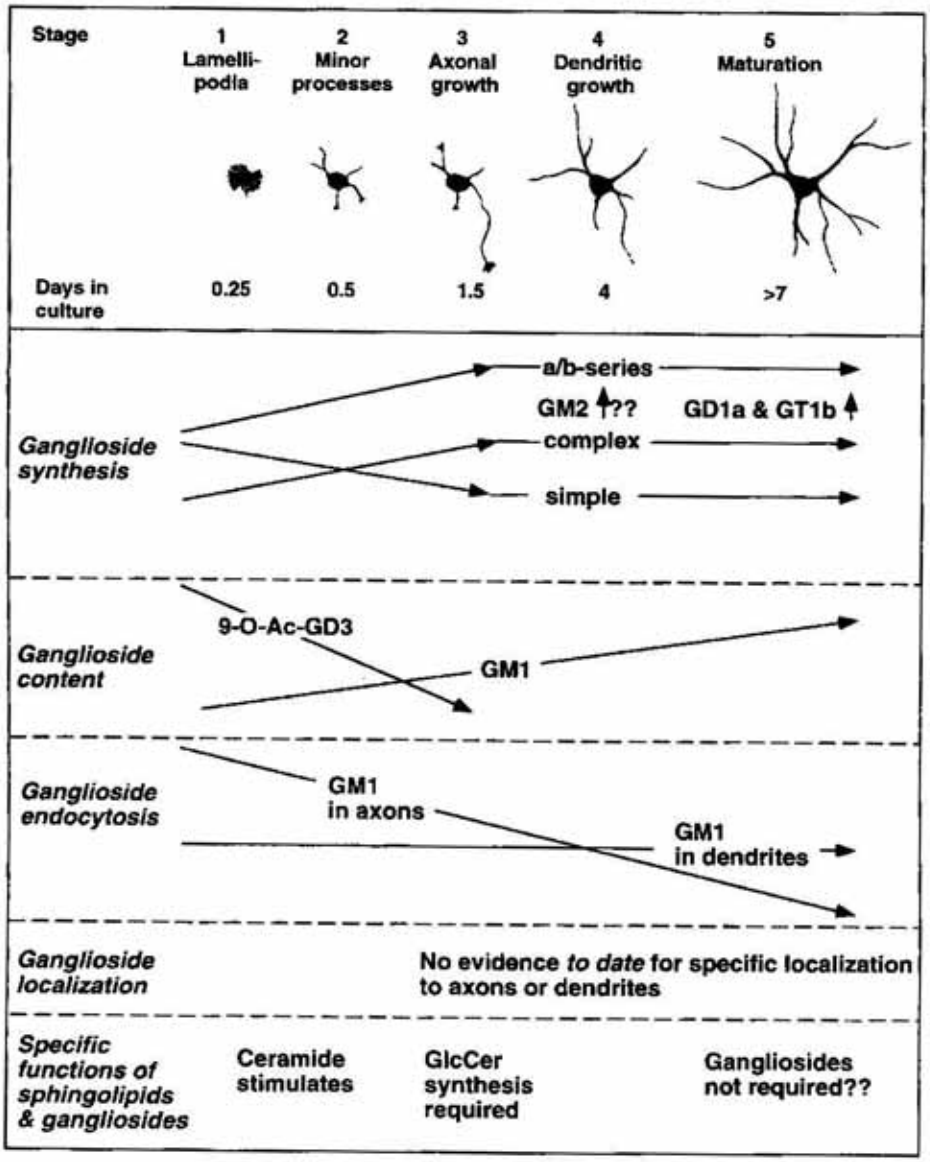

Figure 1. Summary of major findings concerning the roles of sphingolipids and glycosphingolipids in neuronal development.

The stages of development of cultured hippocampal neurons (Dotti *et al.*, 1988) are shown in the top panel. Data concerning ganglioside synthesis, content, endocytosis and localization are discussed in the first part of the text. The second part of the text discusses specific regulatory roles of ceramide and GlcCer at each of the stages of development shown in the top panel.

dritogenesis, dendrite growth and synaptogenesis, there was little change in ganglioside synthesis, with a small and gradual increase in the ratio of a- to b-series gangliosides and an increase in the synthesis of gangliosides G_{D1a} and G_{T1b}. These results demonstrated that changes in ganglioside synthesis were restricted to early stages of neuronal development, namely axonogenesis and rapid axon elongation (Hirschberg *et al.*, 1996) and that few significant changes occurred at later stages. However, one potentially interesting change may occur between days 3 to 8 in culture, the stage at which morphologically distinct dendrites form. Although G_{M2} is a minor ganglioside in hippocampal neurons, its synthesis appeared somewhat elevated during this period, but the generally low levels of synthesis made reliable and accurate quantification difficult. If G_{M2} was indeed elevated, this would lend support to the idea, based on the accumulation of G_{M2} during ectopic dendrite formation in lysosomal storage diseases (Siegel & Walkley, 1994), that G_{M2} plays an important role in dendritogenesis.

We also examined ganglioside content during development. These studies were performed by morphological (immunofluorescence) analyses, as the small amounts of cellular material available in this culture system precludes mass measurement of gangliosides, or of any other lipids. Using a variety of antibodies and ganglioside-binding toxins (e.g. cholera toxin), we showed that, as expected, the number of 9-*O*-acetylated-G_{D3}-positive cells decreased significantly during the transition from stage 2 to stage 3 (e.g. extension of an axon) (Schwarz & Futerman, 1997a). In contrast, the amount of G_{M1} per cell increased about 15-fold during the first two weeks in culture (Sofer & Futerman, 1996). All of our findings concerning ganglioside content were similar to those obtained by other laboratories in other neuronal culture systems.

One of the most fascinating ideas proposed over the past few years relating to ganglioside function was the suggestion that GSLs (and gangliosides) might be preferentially localized to axonal plasma membranes (Dotti *et al.*, 1991), rather than to dendritic plasma membranes. This suggestion was based on the analogy between the sorting and localization of proteins and lipids in epithelial cells and in neurons. Epithelial cells contain two distinct membrane domains, the apical and basolateral membranes, which differ in protein and lipid compositions (Simons & van Meer, 1988). In the early 1990s it was reported that molecules which are sorted to the apical membrane of polarized Madin-Darby Canine Kidney epithelial cells are sorted to the axonal membrane of neurons, and molecules which are sorted to the basolateral membrane of Madin-Darby Canine Kidney cells are sorted to the dendritic membrane of neurons. The generality of this model is at present unclear as a large number of exceptions exist. However, since it was known that certain glycosphingolipids are preferentially sorted to and enriched in the apical domain (Simons & van Meer, 1988), it was proposed that GSLs might be sorted to and enriched in the axonal membrane domain of neurons (Dotti *et al.*, 1991). Various studies from our laboratory, mainly using anti-ganglioside antibodies (Harel & Futerman, 1993; Schwarz & Futerman, 1997a; Sofer & Futerman, 1995), and from others laboratories (Furuya *et al.*, 1995; 1996), do not lend support to this hypothesis, and indeed there is some evidence that the dendrites rather than axons may be actually enriched in certain gangliosides (for review see Schwarz & Futerman, 1996).

A word of caution must be added when discussing determination of the localization of gangliosides using anti-ganglioside antibodies, since the apparent localization of gangliosides can be greatly influenced by the fixation method (Schwarz & Futerman, 1997a). For instance, using monoclonal antibody A2B5 (which reacts with a variety of gangliosides), neurons were labeled at the cell surface when incubated with the antibody prior to fixation, but when incubated post-fixation, cells dis-

played a variety of labeling patterns depending on the fixation method. Biochemical analysis demonstrated that some of the fixatives studied (particularly acetone and methanol) significantly reduced or completely depleted cellular gangliosides, implying that the immunoreactivity observed with A2B5, and with other antibodies, was not due to gangliosides. When hippocampal neurons were incubated with an anti-G_{D1b} antibody pre-fixation, uniform labeling of the plasma membrane was observed, but after ganglioside depletion using biochemical inhibitors of ganglioside synthesis, no cell surface labeling was detected. However, even in cells depleted of gangliosides, labeling of both the cell surface and intracellular compartments was observed when the anti-G_{D1b} antibody was applied post-fixation. In contrast, the JONES antibody (which reacts with 9-*O*-acetylated-G_{D3}), labeled neurons with an essentially similar pattern irrespective of the fixation method. These observations demonstrated that great care must be taken in assigning gangliosides to specific cell populations or to intracellular locations based solely on the use of anti-ganglioside antibodies, and suggest that optimal fixation conditions must be established separately for each anti-ganglioside antibody (Schwarz & Futerman, 1997a).

If dendrites are enriched in certain gangliosides, specific mechanisms must exist to sort and transport them to the dendritic membrane from their sites of synthesis in the cell body, and to target them back to the dendritic membrane after their internalization by endocytosis. We have shown that there is considerable endocytosis of gangliosides from dendrites of hippocampal neurons, at least of ganglioside G_{M1}, at all stages of neuronal development, but that the rate of endocytosis of ganglioside G_{M1} decreases from axons as neurons mature. These studies were performed using either a short acyl-chain fluorescent derivative of ganglioside G_{M1}, *N*-(6-(4-nitrobenz-2-oxa-1,3-diazole-7-yl)-aminohexanoyl)-G_{M1} (Sofer *et al.*, 1996), or using fluorescent derivatives of cholera toxin (Sofer & Futerman, 1996), which binds to endogenous G_{M1}.

In conclusion, we have shown that hippocampal neurons provide an excellent tool to study various aspects of ganglioside biochemistry and cell biology. Moreover, as described below, this culture system has allowed us to define specific and defined *regulatory* roles for the simpler SLs, ceramide and GlcCer, at distinct stages of neuronal development.

REGULATORY ROLES FOR CERAMIDE AND GlcCer IN NEURONAL DEVELOPMENT

We examined whether the initial stages of neuronal development (Fig. 1, stages 1–3) require ongoing synthesis of SLs or GSLs. These studies took advantage of the availability of a number of specific inhibitors of SL and GSL metabolism (for review see Futerman, 1994b). Central to this work was fumonisin B_1 (FB_1), a specific inhibitor of N-acylation of the sphingoid long chain bases, dihydrosphingosine (sphinganine) and sphingosine, to dihydroceramide and ceramide, respectively. We also used D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), which inhibits glucosylation of ceramide to glucosylceramide, and conduritol B-epoxide (CBE), which inhibits the lysosomal degradation of glucosylceramide to ceramide.

Surprisingly, incubation of neurons with either FB_1 or D-PDMP during the first 24 to 48 h in culture had no discernible effect on neuronal development (Harel & Futerman, 1993; Schwarz & Futerman, 1997b; Schwarz *et al.*, 1995), suggesting that SL synthesis is not required for either minor process formation or for the initial stages in axon growth. Presumably neurons contain a sufficiently large intracellular pool of SLs at this stage to sustain growth and do not therefore require ongoing synthesis; this is in contrast to later stages of axonal growth when ongoing synthesis is required (see below). Similarly, a recent study

demonstrated that inhibition of endogenous GSL synthesis had no effect on neurite formation induced by retinoic acid in LAN-B human neuroblastoma cells (Li & Ladisch, 1997). In contrast, exogenously added short-acyl chain analogues of ceramide (C_6-NBD-ceramide or C_6-ceramide) stimulated the transition of neurons from stage 1 to 2 (Schwarz & Futerman, 1997b). Analysis of the effects of ceramide stereoisomers on axon growth during the first 24 h in culture were consistent with the notion that ceramide may mediate its effects at this stage of development *via* a signaling pathway. Naturally occurring ceramide occurs in the D-*erythro*- configuration, but three other stereoisomers exist, all of which are active to some extent in ceramide-mediated signaling pathways (Bielawska *et al.*, 1993; Fishbein *et al.*, 1993). Both C_6-NBD-D-*erythro*-Cer and C_6-NBD-L-*threo*-Cer stimulated growth between days 0 and 1, but C_6-D-*erythro*-dihydroCer, which is totally inactive in signaling pathways, had no effect (Schwarz & Futerman, 1997b). Moreover, exogenously added neutral-sphingomyelinase (N-SMase) also enhanced the rate of neuronal development during the first 24 h in culture (Schwarz & Futerman, 1997b).

In proliferating cells, ceramide suppresses growth and stimulates differentiation (Pushkareva *et al.*, 1995). For instance, ceramide inhibits proliferation of neuroblastoma Neuro2a cells and induces their differentiation (Riboni *et al.*, 1995), and stimulates T9 glioma differentiation and process formation (Dobrowsky *et al.*, 1994). However, ceramide cannot act *via* arrest of proliferation and stimulation of differentiation in hippocampal neurons since these neurons are post-mitotic at their time of removal from the hippocampus. Rather, ceramide acts by accelerating the transition from stage 1 to 2.

At present, we do not know the molecular mechanisms by which ceramide accelerates the transition from stage 1 to 2, nor do we know whether an endogenous N-SMase activity is involved in the physiological regulation of this early stage of development. However, using a short-acyl chain derivative of sphingomyelin, C_6-NBD-SM to detect activity (Futerman *et al.*, 1990), we have demonstrated that hippocampal neurons contain significant levels of endogenous N-SMase activity (A. Brann & A. Futerman, unpublished observations). Moreover, neuronal development can be stimulated by various neurotrophins, including NGF and BDNF. Interestingly, binding of neurotrophins to the $p75^{NTR}$ receptor results in generation of ceramide (Dobrowsky *et al.*, 1994), that may in turn (*via* an as yet unknown mechanism) induce gene expression. RNAse protection assays demonstrate that hippocampal neurons cultured according to the method described above express the $p75^{NTR}$ receptor (A. Brann, M. Fainzilber & A.H. Futerman, unpublished observations) and we are currently examining whether this pathway is involved in the acceleration of neuronal development induced by ceramide, and whether a down-stream effect results in alterations in cytoskeletal organization and consequently minor process formation.

Whereas ongoing GSL synthesis is not required for the earliest stages of neuronal development, it is required for normal axonal growth in cultured hippocampal neurons. Initially, we demonstrated that incubation with FB_1 completely blocked axon growth between days 2 and 3 in culture (Harel & Futerman, 1993). This was the first demonstration that axonal growth could be regulated by SL synthesis, although an earlier study had demonstrated an inhibitory effect of D-PDMP on neurite growth in a murine neuroblastoma cell line (Uemura *et al.*, 1991). Subsequently, we demonstrated that D-PDMP also blocked axonal growth in hippocampal neurons. The effects of incubation with D-PDMP were indistinguishable from those of FB_1 (Schwarz *et al.*, 1995). Statistical analyses of various parameters of neuronal growth suggested that the formation or stabilization of new collateral axonal branches was particularly sensitive to manipulating levels of SL synthesis,

and that it was this specific facet of neuronal growth that required ongoing SL synthesis. In contrast to the effects of D-PDMP and FB_1, incubation with CBE (an inhibitor of GlcCer degradation) stimulated axonal growth by enhancing either the rate of formation or stabilization of axonal branches. The stimulatory effect of CBE could be completely abolished by co-incubation with FB_1, implying that the ability of CBE to enhance axonal growth was a result of accumulation of a newly-synthesized GSL or of GlcCer (Schwarz *et al.*, 1995).

It should be emphasized that the reduction in the rate of axonal growth upon inhibition of GSL synthesis was not due to a reduction in the total mass of GSLs, or of GlcCer, since short incubations with inhibitors (of between 1–3 h) (Boldin & Futerman, 1997; Schwarz *et al.*, 1995) were able to block growth. We suggested that the reduction in axon growth was directly related to the synthesis and delivery of newly synthesized GSLs to the axonal membrane (see Futerman & Banker, 1996).

Studies within the past year or two have unambiguously shown that ongoing synthesis of GlcCer is required for axon growth during stage 3 and for accelerated axon growth upon incubation with growth factors. This was demonstrated most clearly in studies using either laminin or basic fibroblast growth factor (bFGF) (Boldin & Futerman, 1997). Both bFGF and laminin stimulate axonal growth about 4-fold. Remarkably, the stimulatory effect of either factor can be completely abolished by incubation with either FB_1 or PDMP. However, addition of C_6-NBD-D-*erythro*-Cer together with FB_1 antagonized the inhibitory effects of FB_1 on bFGF-stimulated growth. In contrast, C_6-NBD-L-*threo*-Cer was totally ineffective in antagonizing the effects of FB_1 and neither C_6-NBD-D-*erythro*-Cer nor C_6-NBD-L-*threo*-Cer were able to antagonize the inhibitory effects of PDMP on bFGF-stimulated growth. Analysis of the metabolism of the ceramide stereoisomers by TLC demonstrated that about 10–15% of C_6-NBD-D-*erythro*-Cer was converted to C_6-NBD-D-*erythro*-GlcCer during a three hour incubation, but C_6-NBD-L-*threo*-Cer was not metabolized at all to C_6-NBD-L-*threo*-GlcCer (Boldin & Futerman, 1997). These data demonstrate that the ability of bFGF and laminin to stimulate axonal growth requires the ongoing synthesis of GlcCer from ceramide. Likewise, normal axon growth between days 2 and 3 in culture also requires the metabolism of ceramide to GlcCer (Schwarz & Futerman, 1997b). These data also demonstrate that the ability of exogenously added ceramide to antagonize the inhibitory effects of FB_1 is not due to activation of a ceramide-mediated signaling pathway, since both D-*erythro*-Cer and L-*threo*-Cer are equally effective in ceramide-mediated signaling pathways (Bielawska *et al.*, 1993; Fishbein *et al.*, 1993), but only C_6-NBD-D-*erythro*-Cer is able to antagonize the effects of FB_1. Moreover, since C_6-NBD-D-*erythro*-Cer is only metabolized to C_6-NBD-D-*erythro*-GlcCer, and not to higher order complex GSLs in cultured hippocampal neurons (Boldin & Futerman, 1997; Harel & Futerman, 1993), our data also show that ongoing synthesis of GlcCer, but not of higher-order glycosphingolipids, is required for bFGF to stimulate axon growth.

Two general possibilities could explain the need for GlcCer synthesis in axonal growth. First, GlcCer might be required for bFGF and laminin to transduce signals after they bind to cell surface receptors; however, this possibility is unlikely since levels of cell surface GlcCer will not change significantly during a 3 h incubation with FB_1 or PDMP (see Schwarz *et al.*, 1995). The second possibility is that the requirement for ongoing GlcCer synthesis is related to the need to continually supply newly-synthesized GlcCer from its site of intracellular synthesis to the growing axonal membrane. In this scenario, the requirement for GlcCer synthesis could either be related to an event occurring at its site of synthesis, or alternatively, could be related to an event occurring at the axonal plasma membrane after GlcCer is inserted into the membrane. In non-neuronal cells, it has been shown that GlcCer

is synthesized in a pre- or early Golgi apparatus compartment (Futerman, 1994a), which in neurons is restricted to the cell body and proximal dendrites (see Futerman & Banker, 1996). Inhibition of GlcCer synthesis might directly affect the formation of Golgi-apparatus derived vesicles, if GlcCer is a rate-limiting component in vesicle formation. However, recent studies have shown that both C_6-NBD-D-*erythro*-Cer and C_6-NBD-L-*threo*-Cer are found in Golgi-apparatus derived vesicles in hippocampal neurons (M.. Burack, G. Banker & A. Futerman, unpublished observations), even though only C_6-NBD-D-*erythro*-Cer is metabolized to GlcCer and able to reverse the inhibitory effects of FB_1 on axonal growth. Alternatively, GlcCer synthesis might be required for the transport and insertion into Golgi-derived vesicles of a key protein that is involved in regulating axonal growth. In Chinese hamster ovary cells, inhibition of GlcCer synthesis blocks the delivery of a viral protein to the cell surface (Rosenwald *et al.*, 1992), although these effects were subsequently attributed to the accumulation of ceramide (Chen *et al.*, 1995; Rosenwald & Pagano, 1993) and not to the depletion of GlcCer. This cannot be the reason for the disruption of axonal growth observed in hippocampal neurons since exogenously added ceramide does not disrupt growth at this stage of development, and ceramide must be metabolized to GlcCer to support growth. We are currently examining the transport and delivery of two viral proteins through the secretory pathways to the cell surface with and without inhibitors of SL synthesis at different stages of axonal growth.

One prediction of our studies is that stimulation of axonal outgrowth by either bFGF or laminin may stimulate GlcCer synthesis, assuming that GlcCer synthesis is a rate-limiting step in axonal growth. Studies are presently underway to test this prediction, and preliminary data indicate that the rate of GlcCer synthesis is increased about 2-fold *in vivo* upon incubation of hippocampal neurons with either bFGF or laminin (S. Boldin & A. Futerman, unpublished observations). If these experiments are validated, they will imply that GlcCer synthase is a regulated enzyme, and moreover, since regulation of enzyme activity occurs within short time periods, regulation must occur post-translationally, rather than by up-regulation of protein synthesis. Interestingly, incubation with either cycloheximide (which inhibits protein synthesis) or brefeldin A (which disrupts the Golgi apparatus and consequently blocks Golgi-derived vesicle traffic) also blocks the ability of bFGF and laminin to stimulate axonal growth (S. Boldin & A. Futerman, unpublished observations), although the cause of this block is presumably due to a reduction in bulk membrane components rather than a reduction in a specific lipid, as is the case upon incubation with either FB_1 or PDMP.

To date, I have discussed data demonstrating that ceramide and GlcCer are involved in regulating two distinct processes, minor process formation (by ceramide) and axon growth (by GlcCer). Our work has also shown that ceramide is involved in a third distinct process, namely apoptosis. Many previous studies demonstrated that generation of ceramide by incubation with N-SMase, or by addition of short-acyl chain analogues of ceramide, induce apoptotic cell death (Hannun & Obeid, 1995). In hippocampal neurons, neither C_6-NBD-D-*erythro*-Cer nor N-SMase affected cell viability at low concentrations (see above), but, at higher concentrations, both induced cell death at early (stage 1 and 2) and at later (stage 3) periods of development, as determined by analysis of chromatin condensation and annexin V-binding (Schwarz & Futerman, 1997b). C_6-L-*threo*-Cer also induced apoptosis, but C_6-D-*erythro*-dihydroCer had no effect on cell viability at any stage of development. The lack of specificity of ceramide stereoisomers in inducing apoptosis and in regulating the transition from stage 1 to 2, together with the lack of effect of dihydroceramide (Bielawska *et al.*, 1993), is consistent with the possibility

that ceramide acts *via* an intracellular signaling pathway in the regulation of these events.

CONCLUDING REMARKS

Our studies on SLs and GSLs in polarized neurons have helped define the regulatory roles that these lipids play in neuronal growth and development. Determination of the molecular mechanisms by which these lipids regulate development is currently the subject of intense research in our laboratory. In contrast, we are unable at present to ascribe precise roles to gangliosides. It seems unlikely that neurons would synthesize different gangliosides at different stages of development (particularly during axon growth) unless they served specific and defined functions, but it has proved difficult to ascertain these functions. Describing the roles of gangliosides, and their modes of intracellular transport in polarized neurons is also currently the focus of an intensive research effort in our laboratory.

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