Ganglioside Function in the Development and Repair of the Nervous System

From Basic Science To Clinical Application

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Abstract

Gangliosides play important roles in the normal physiological operations of the nervous system, in particular that of the brain. Changes in ganglioside composition occur in the mammalian brain not only during development, but also in aging and in several neuropathological situations. Gangliosides may modulate the ability of the brain to modify its response to cues or signals from the microenvironment. For example, cultured neurons are known to respond to exogenous ganglioside with changes characteristic of cell differentiation. Gangliosides can amplify the responses of neurons to extrinsic protein factors (neuronotrophic factors) that are normal constituents of the neuron's environment. The systemic administration of monosialoganglioside also potentiates trophic actions in vivo and improves neural responses following various types of injury to the adult mammalian central nervous system. The possible molecular mechanism(s) underlying the ganglioside effects may reflect an action in modulating ligand-receptor linked transfer of information across the plasma membrane of the cell.

Index Entries: Gangliosides; nervous system; neuroplasticity; cell differentiation; neuronotrophic factors; microenvironment; neuronotoxic influences; neurodegeneration; aging; regeneration; ligand-receptor information transfer.

Introduction

Gangliosides have generated considerable interest among neurobiologists for some time, owing to their unusual physicochemical properties and high concentration in the brain. Early studies included demonstrations that gangliosides could restore excitability of brain slices to electrical pulses (McIlwain, 1963) and that antibody against brain gangliosides could elicit epileptic seizures when applied locally to the brain surface (Karpiak et al., 1976). Even so, the physiological function of gangliosides, especially with respect to the nervous system, remained largely a matter of speculation.

Use of both tissue culture techniques for neural cells and in vivo experimental paradigms has now made it possible to explore the question of ganglioside action. Among the numerous functions proposed for gangliosides are those concerned with promotion of neuritogenesis and neuronal differentiation, axonal regeneration, and modification of neuronotrophic and neuronotoxic influences. These developments have provided the impetus for considering the efficacy of gangliosides as potential therapeutic agents for neuropathological conditions.

Some Basic Chemical-Biological Considerations of Gangliosides

Gangliosides are glycoconjugates consisting of sialooligosaccharides linked to a ceramide moiety (Ledeen, 1983; Svennerholm, 1984; Wiegandt, 1982). They represent a unique class of molecules that are negatively charged, amphipathic, and that have a diversity of structures. Over 60 molecular species of gangliosides have been isolated, from the simple sialylgalactosylceramide (GM4) to complex fucosyl- and polysialogangliosides. The ceramide consists of a long-chain fatty acid linked by an amide bond to a long-chain base, unsaturated or saturated. The sialosyloligosaccharide is β-glycosidically linked to the ceramide, and consists of a neutral oligosaccharide core to which variable numbers of sialic acid residues are attached. Figure 1 illustrates the structure of the monosialoganglioside GM1. The structural classification of gangliosides follows that according to Svennerholm (1963).

The highest concentrations of gangliosides in mammals are found in the gray matter of the nervous system, in contrast to other glycosphingolipids (Ando, 1983; Ledeen, 1983,1985). The

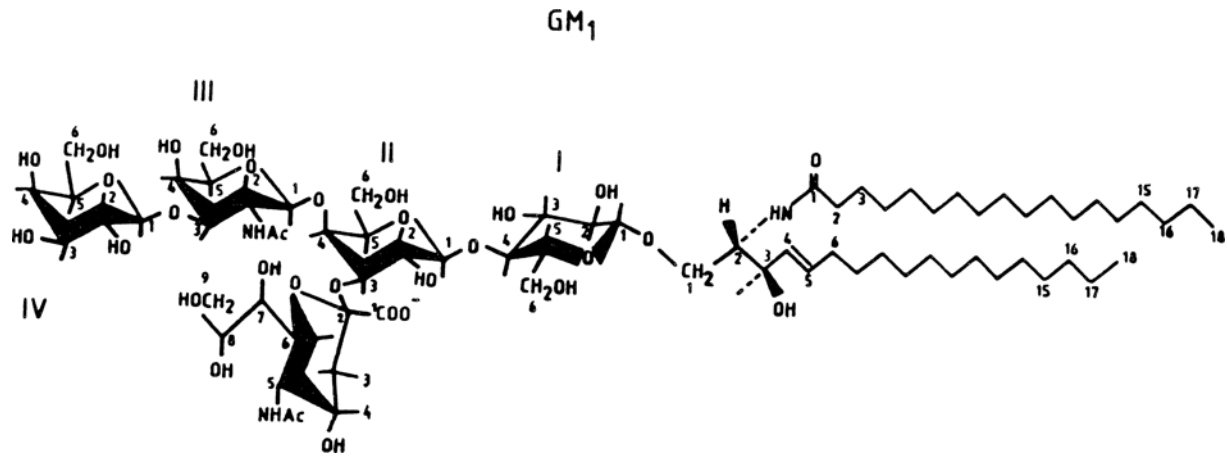

Fig. 1. Chemical structure of GM1 ganglioside.

gangliosides found in this region differ qualitatively from those found in other tissues, as well as within the same tissue. For example, the gangliosides present in the central nervous system (CNS) are mostly of the ganglio series, whereas those of the peripheral nervous system (PNS) and extra-neuronal tissues contain high amounts of the lacto- and globo-series gangliosides.

Gangliosides are synthesized in the cytosol and transported to the plasma membrane, where they are almost exclusively localized to the outer leaflet of the lipid bilayer. The ceramide portion is inserted into the lipid bilayer, whereas the sialosyloligisaccharide head group protrudes toward the external mileau. Gangliosides comprise a major part of the glyconjugate network, extending from the neuronal membrane surface, with high concentrations having been found in synaptic plasma membranes. The degree of uniformity of their distribution over the plasma membrane remains a question, however. Because of their location and diversity of structure, gangliosides are well-suited to function as cell surface receptors and as modulators of various membrane processes.

Ganglioside Expression in Neural Development

Molecules that may be involved in developmentally regulated cellular and cell-substratum interactions important for neuronal development include glycolipids, particularly gangliosides, and glycoproteins. Impetus for this assumption dates back to the original discovery of gangliosides in the human brain by Klenk (1942), and of inherited defects of ganglioside metabolism (Sandhoff and Christomanou, 1979) leading to abnormal brain development.

Different brain areas would be expected to differ with respect to developmental profiles of gangliosides (Hilbig et al., 1983,1984; Rösner, 1977; Seybold and Rahmann, 1985; Vanier et al., 1971), with different neuronal and glial cell types containing specific and characteristic sets of gangliosides (Byrne et al., 1988; Dreyfus et al., 1980; Kim et al., 1986). Some general principles of an empirical nature, however, are evident in the developmental regulation of ganglioside expression in the brain. These principles are depicted in Fig. 2, and are briefly summarized below (cf., Rösner and Rahmann, 1987).

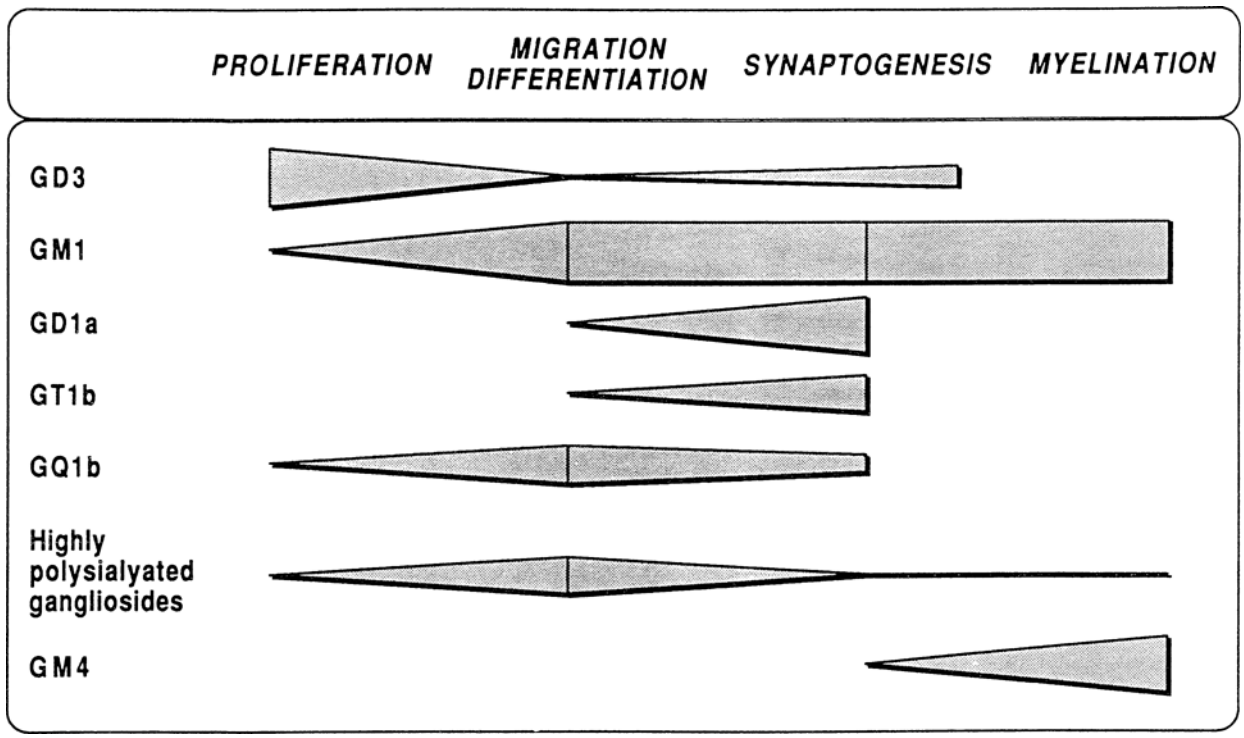

Fig. 2. Gangliosides in neuronal development of higher vertebrates.

Proliferating neuronal and glial precursor cells express a simple set of gangliosides, predominantly GD3 and GM3 (Goldman et al., 1984; Sbaschnig-Agler et al., 1988); more complex gangliosides containing a complete tetraose moiety are generally absent. After neuroblast mitosis concludes, GD3 content decreases, with the post-mitotic neurons now accumulating highly sialylated complex gangliosides (Rösner, 1980,1982). During the growth spurt period synaptogenesis commences, accompanied by a marked increase in neuron size. A several-fold increase in ganglioside synthesis follows, with a rapid accretion of gangliosides synthesized via the a-pathway, like GM1 and GD1a, in addition to gangliosides derived via those of the b-pathway, like GD1b and GT1b (Rösner, 1980; Willinger and Schachner, 1980); concurrently, expression of polysialogangliosides of the c-pathway is decreased (*see* Tettamanti et al., (1987) for a discussion of the lines of biosynthesis of ganglioseries gangliosides). The onset of myelination leads to an increasing accumulation of GM1 and GM4. This last developmental period, which continues into adulthood, is preceded by a second rise of GD3, most likely representing proliferation of oligodendroglia. The relative amounts of b-pathway gangliosides increase in adulthood, compared to those of the a-pathway (Kracun et al., 1986). During aging, total ganglioside-bound sialic acid markedly decreases, owing mainly to a further loss of GD1a (Ando et al., 1986).

Neurological Disorders and Mutations Related to Defects of Ganglioside Metabolism

Normal developmental changes in the ganglioside profile of the nervous system may be re-

lated to proliferation, neuronal differentiation, or myelinogenesis. At the same time, several ganglioside storage diseases are known in humans: GM1-gangliosidosis (β-galactosidase deficiency) and GM2 gangliosidosis (Tay-Sachs disease and its β-hexosaminidase-deficient variants (O'Brien, 1983)). In both diseases, the major pathological characteristic is the lysosomal accumulation of gangliosides in neurons. Ultrastructural studies of neurons in humans and animals with GM1- and GM2-gangliosidosis have revealed aberrant sprouting of neurites from axon hillocks (meganeurites) prior to neuronal death (Purpura and Suzuki, 1976; Purpura and Baker, 1978).

These metabolic disorders can be used to study the relative susceptibility of neuronal populations to abnormal ganglioside catabolism. A number of patients with the β-hexosaminidase variant of GM2-gangliosidosis display cerebellar atrophy and motor neuron disease (Johnson, 1981), suggesting that these populations of neurons either metabolize gangliosides more slowly than others (e.g., retinal ganglia) or are more sensitive to ganglioside accumulation. GM2-gangliosidosis variants can also include the absence of a GM2-activator protein (transfer protein) (Hinrichs et al., 1986). In feline GM1-gangliosidosis, major alterations of cholinergic function have been found in the brain of this animal model (Jope et al., 1985).

A large number of mouse strains displaying inherited neurological abnormalities have been described. In mutations affecting CNS myelination, neuraminic acid content is reduced, especially in the jumpy mutant (Baumann et al., 1980). In the quaking mutant brain, which is characterized by an excess of polysialogangliosides and a defect in myelin compaction, a pool of GM1 linked to myelin is markedly reduced (Baumann et al., 1987). Mutations affecting cerebellar development have also been studied. A considerable reduction of GT1a has been reported in Purkinje cell degeneration, suggesting that this ganglioside is primarily a neuronal constituent enriched in Purkinje cells (Seyfried et al., 1982). Although it is difficult to always correlate such molecular changes to abnormal developmental processes, mutations can still provide a useful paradigm for studying the sequential phases of nervous system development under the influence of genetic alterations. Table 1 summarizes some of these defects.

Antiganglioside Antibodies and Neurodegenerative Diseases

Studies on ganglioside content and metabolism have provided much information on their role during nervous system development. Other insights may derive from neuropathological situations that appear to be associated with antiganglioside antibodies. Recent studies have demonstrated that gangliosides or other glycolipid antigens react with human IgM monoclonal antibodies in the paraproteinemic neuropathies in which demyelination is often a prominent feature (Chou et al., 1986; Ilyas et al., 1984,1985; Quarles et al., 1986). Serum IgM antibodies reacting with GD1a and GT1b gangliosides have been reported in patients with Guillain-Barré syndrome, an inflammatory demyelinating polyneuropathy (Ilyas et al., 1988a). Polyneuropathies associated with gammopathy have also been described, in which IgM monoclonal antibodies recognize the gangliosides GM1 (Ilyas et al., 1988b), or GM2, IV^4Ga1N-AcGM1b and IV^4Ga1NAcGD1a (Ilyas et al., 1988c). In at least one case of motor neuron syndrome, a monoclonal IgM was demonstrated to have antibody activity against gangliosides GM1 and GD1b (Nardelli et al., 1988).

High levels of antibodies to gangliosides GM1 but not to other gangliosides (GD1a, GD1b, GT1b, and GQ1b) have been reported in patients with Alzheimer's disease, and in pa-

Table 1
Genetic Mutant Analysis in the Cellular Expression of Brain Gangliosides

Cell defects	
Purkinje cell degeneration (pcd/pcd)—GT1a deficiency	
Stagger (sg/sg)	
Lurcher mutant (Lc/+)	
Catabolic defects	
Ganglioside storage diseases; GM1-, GM2-gangliosidoses (meganeurites)	
Anabolic defects	
t^{w1} mouse mutation—GQ1 deficiency in embryos with failure of neuronal differentiation in neural tubes	

tients with Parkinson's disease with dementia but not in nondemented patients (Chapman et al., 1988). This last observation is of special interest, because of the possible clinical overlaps between Parkinson's disease with dementia and Alzheimer's disease (Quinn et al., 1986). The pathological significance of antiganglioside antibodies remains to be established; however, antibodies with at least some of these specificities can cause neuronal damage and/or dysfunction in vitro or in in vivo test systems (*see* the following section).

In Vitro Responses of Neuronal Cells to Gangliosides: The Role of Neuronotrophic Factors

Implication of Gangliosides as Neuritogenic Agents

A role for gangliosides, and, in particular, GM1, in axonal growth has been suggested from several types of studies. The expression of GM1 on maturing postmitotic cerebellar granule neurons and their growing neurites gradually becomes restricted to the cell body as the neuron matures (Willinger and Schachner, 1980). Ultrastructural observations of mature neurons in humans and cats with genetically determined ganglioside storage diseases (GM1- and GM2-gangliosidosis), which describe aberrant sprouting of neurites from affected neurons (Purpura, 1978; Purpura and Baker, 1977; Purpura and Suzuki, 1976) have been interpreted as evidence of an effect of altered cell surface ganglioside patterns, with the overly abundant ganglioside acting as a possible neuritogenic agent. Furthermore, anti-GM1 antibodies inhibit neurite regeneration in vitro (Spirman et al., 1982) and axonal elongation in vivo (Sparrow et al., 1984), as well as produce long-lasting morphological and behavioral abnormalities when administered to developing animals (Kasarskis et al., 1981).

Neuroblastoma Cells

Neuroblastoma cells have been frequently utilized as a model system for examining the role(s) of plasma membrane constituents in the regulation of neurite outgrowth and cellular differentiation. Under some conditions, these cells may form neurite-like processes and express biochemical properties characteristic of mature neurons (de Laat and van der Saag, 1982). Several neuroblastoma cell lines undergo morphological differentiation following addition to their culture medium of different ganglioside species, including GM1 (Fig. 3) (Leon et al., 1982; Morgan and Seifert, 1979; Tsuji et al., 1983). In N_2A cells, the GM1 effect is correlated with a stable insertion of the ganglioside into the plasma membrane (Facci et al., 1984) and is ac-

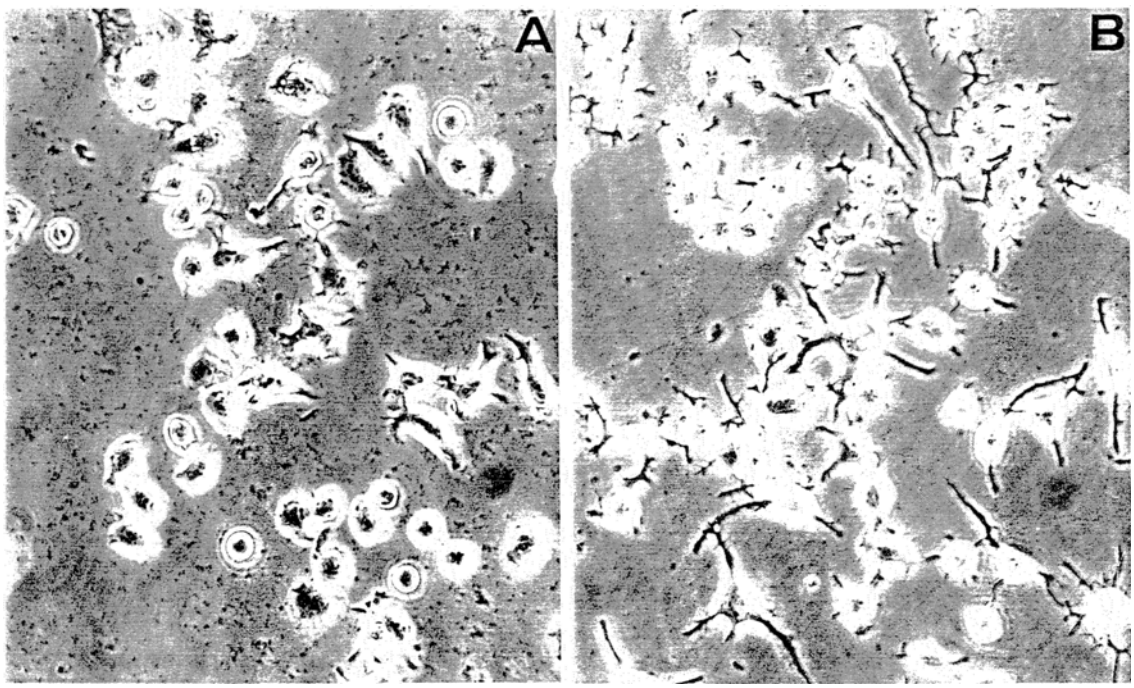

Fig. 3. Neuroblastoma N_2A cells grown for 24 h in the absence (left) or presence of 100 μ*M* GM1 (right).

companied by increases in cyclic AMP content and protein phosphorylation (Roisen et al., 1986). In the neural hybrid clonal cell line SB21B1, ganglioside-induced neurite outgrowth is followed by an increased expression of mRNA for tubulin (Rybak et al., 1983).

Neuronotrophic Factor-Responsive Neuronal Cells

Extracellular influences are important for neuritogenesis in development and/or neurite repair in the adult. The potential for gangliosides to modulate the behaviors of neuronal cells in response to cues or signals from the microenvironment must also be considered. Several specific examples will be given to illustrate how gangliosides can potentiate the responses of neurons to extrinsic protein factors that are normal constituents of the neuronal environment, the latter agents being neuronotrophic factors.

Information concerning neuronotrophic factors derives, in large part, from the discovery and investigation of the Nerve Growth Factor (NGF). Trophic factors are special proteins produced by the innervation territories of neurons, where they are taken up by nerve terminals and retrogradely transported along the axon to the cell body, to carry out their biological actions. The work of Levi-Montalcini (Levi-Montalcini, 1966, 1987) demonstrated that NGF exerts trophic control of neural crest-derived sensory, and sympathetic neurons. Nerve growth factor may also provide a trophic function for some cholinergic neurons of the CNS (cf., Korsching, 1986). Several other macromolecules with neuronotrophic activity have been purified since the identification of NGF. Among these are Ciliary Neuronotrophic Factors (CNTF) (Barbin et al., 1984; Manthorpe et al., 1986; Watters and Hendry, 1987) and Brain-Derived Neurotrophic Factor (Barde et al., 1982).

Numerous findings support a neuritogenic effect of gangliosides in relationship to the presence of trophic influences. The addition of ganglioside GM1 to the culture medium of chicken embryonic d 8 (E8) dorsal root ganglia or E11 sympathetic ganglia, under the appropriate culture conditions facilitates NGF-induced neurite outgrowth (Leon et al., 1984; Roisen et al., 1981; Skaper and Varon, 1985) (Fig.4). Similar results have also been observed in the corresponding dissociated primary neuronal cell cultures (Doherty et al., 1985; Leon et al., 1984; Skaper et al., 1985) (Fig. 5), with a proper balance between permissive and inhibitory influences providing for an optimal response (Skaper et al., 1985)—as also observed for the responses with ganglia (Skaper and Varon, 1985). GM1 has also been reported to enhance NGF effects in adult mouse sympathetic ganglia (Spoerri, 1986), indicating no limitation to fetal tissue. More importantly, the ability of GM1 to potentiate neuronotrophic action is not limited to NGF, but is also effective with parasympathetic neurons and CNTF (Skaper et al., 1985), and dorsal root ganglia and NGF-unlike trophic activities in cell conditioned medium (Spoerri and Roisen, 1988). Antibodies to GM1 have been shown to block both NGF (Schwartz and Spirman, 1982) and conditioned media (Spoerri et al., 1988) induced neuritogenesis of chicken embryo sensory ganglia, suggesting that endogenously occurring GM1 molecules may play a role in mediating the trophic effects.

The rat pheochromocytoma cell line PC12 can be used to demonstrate an interesting aspect of GM1 potentiation of trophic factor-dependent events. Unlike primary neurons, PC12 cells respond to NGF with expression of properties characteristic of mature sympathetic neurons, without requiring NGF for survival (Greene and Shooter, 1980). Ganglioside GM1 has been shown to elicit neurites from PC12 cells first primed with NGF (Ferrari et al., 1983) and from NGF naive PC12 cells (Ferrari et al., 1983; Katoh-Semba et al., 1984), but only when the cells have been treated with GM1 and NGF together. Similarly, older chicken embryonic (E15) sensory neurons, that survive without NGF but that are NGF responsive in terms of neurite outgrowth, still require NGF for the ganglioside effect (Skaper et al., 1985).

The addition of gangliosides, including GM1, to a variety of primary dissociated CNS neurons in vitro has been reported to facilitate neurite outgrowth, as well (Massarelli et al., 1985; Skaper et al., 1985). Specific neuronotrophic protein molecules have not been established for these cells, but required low molecular weight trophic agents have been identified (Selak et al., 1985). Addition of GM1 to cultured fetal mouse mesencephalic cells enhanced the biochemical development and survival of the dopaminergic and GABAergic neurons (Leon et al., 1988), effects that were correlated with the stable membrane insertion of the ganglioside molecules and the presence of cell density-derived trophic influences.

These neuritogenic effects of ganglioside require the integrity of the GM1 molecule, as asialo GM1 (lacking sialic acid) has no activity. Other ganglioside species are reported to be functional. Among them, the major bovine brain ganglioside (GD1a, GD1b, and GT1b) were effective only in the presence of NGF, in PC12 cells and E8 chicken sensory neurons (Doherty et al., 1986; Ferrari et al., 1983). Using again PC12 cells and sensory neurons, epi-GM3 (a synthetic epimer of GM3 having a neuraminidase-resistant β-ketosidic linkage) (Cannella et al., 1988a) and a glycero-ganglioside (a glycerol-containing analog of ganglioside, with sialic acid attached to a diglyceride-like structure having two ether-linked alkyl chains) (Cannella et al., 1988b) stimulated neurite outgrowth, indicating that metabolism of the sialic acid group of exogenous ganglioside is not directly involved in the neuritogenic process, while stressing the importance of this moiety.

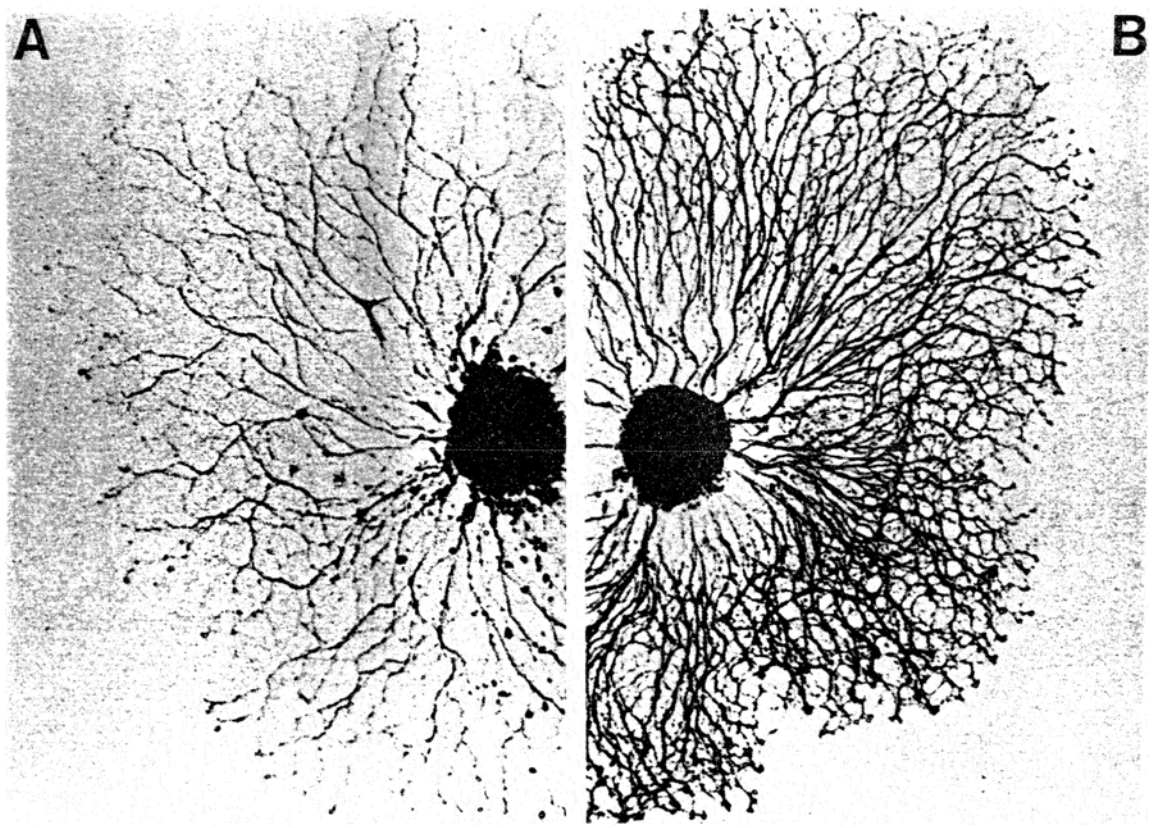

Fig. 4. GM1 ganglioside potentiates the effect of NGF on neurite outgrowth from chicken E11 sympathetic ganglia. Ganglia were grown for 48 h with NGF (10 ng/mL) without (A) or with 100 μM GM1 (B).

The above in vitro studies bring out two very important points concerning ganglioside action. First, in neuronal cells with a trophic factor requirement for survival and/or neurite outgrowth, ganglioside will not substitute for the trophic factor, but will only potentiate the response of the cell to the trophic factor. Second, the neuritogenic response to ganglioside is a time-related gain; in other words, one observes that neurite outgrowth begins faster with ganglioside present. However, the number of neurite-bearing cells will, ultimately, not be different (Katoh-Semba et al., 1984; Skaper et al., 1985). Gangliosides, thus, seem to modulate the execution of a neurite program, rather than actually initiating the program itself. It is important to emphasize that ganglioside effects in the presence of neuronotrophic factors necessitate a balance between neurite permissive and inhibitory influences that, in turn, permits a positive ganglioside effect. This principle is a critical one, in that neuronal damage and/or death in the adult following traumatic injury or pathological events or aging may also reflect a balance between diverse environmental signals, and could conceivably determine the ability of exogenous ganglioside to exert a beneficial effect.

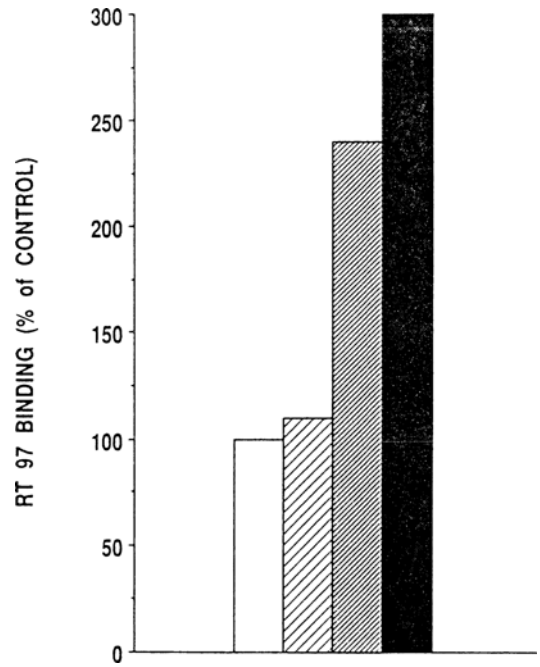

Fig. 5. Effects of NGF and/or GM1 on the binding of RT97 to sensory neurons. Dorsal root ganglion cells were cultured for 2 d with NGF (5 ng/mL) ± GM1 (100 μM). Neurite regeneration was quantitated by measuring the binding of a neurofilament protein monoclonal antibody (McAbRT 97) with an ELISA technique (Doherty et al., 1985). The GM1 potentiation of NGF-induced increase in neurofilament protein expression was statistically significant ($p < 0.01$, Student's *t*-test).

Control (□), GM1 (▨), NGF (▨), NGF + GM1 (■).

Gangliosides and Functional Recovery of the Damaged Nervous System

Understanding the mechanisms underlying neuronal plasticity has benefited greatly from cellular studies at the in vitro level. Identification of molecules that can influence the way neurons respond to extracellular signals may facilitate attempts at repair of a damaged nervous system. The importance of in vitro models cannot be overemphasized, but in vivo observations are needed to validate the former.

Studies from many independent laboratories have shown that exogenously administered gangliosides are effective in enhancing neuronal repair in experimental in vivo paradigms mimicking pathological situations of either the peripheral nervous system (PNS) or CNS. An overview of the current information on this subject is presented below (*see also* Mahadik and Karpiak, 1988; Stein and Sabel, 1988).

Ganglioside Treatment and PNS Repair Processes

The first indication that exogenous gangliosides can facilitate nervous system repair in vivo appeared in 1976. Ceccarelli et al. (1976) demonstrated that the parenteral administration of a bovine brain ganglioside mixture was

capable of enhancing recovery of the denervated-nictating membrane in the cat. This effect was independent of the specific neurons involved, i.e., cholinergic or adrenergic (following either pre- or post-ganglionic denervation, respectively). A similar effect was observed with the extensor digitorum muscle of the rat (Gorio et al., 1980). Numerous, independent reports have supported the conclusion that gangliosides are effective in improving repair processes in various models of peripheral nerve damage: traumatic, metabolic, or toxic (Calcutt et al., 1988; Gorio et al., 1983; Kalia and Di Palma, 1982; Kleinbeckel, 1982; Marini et al., 1986; Norido et al., 1981,1984; Robb and Keynes, 1984; Sparrow and Grafstein, 1982).

Ganglioside Treatment and CNS Repair Processes

The potentiating role of gangliosides, in particular GM1, administration on postlesion recovery in the CNS is well-documented. GM1-induced improvements of biochemical, morphological, and behavioral parameters after various types of brain lesion (mechanical, chemical, electrolytic) have been observed. The first reports described enhanced survival and function of lesioned nigral dopaminergic neurons following GM1 treatment (Agnati et al., 1983; Toffano et al., 1983). Subsequent studies demonstrated that administration of GM1 ganglioside stimulated the recovery of dopaminergic (Agnati et al., 1985; Commissiong and Toffano, 1986; Kojima et al., 1984; Raiteri et al., 1988; Toffano et al., 1984a, b; Yavin et al., 1987), serotonergic (Fusco et al., 1986,1988; Hadjiconstantinou and Neff, 1986; Jonsson et al., 1984), and cholinergic (Casamenti et al., 1985; Cuello et al., 1986; Oderfeld-Nowak et al., 1984; Sofroniew et al., 1986) neurons. GM1 treatment has been reported to also facilitate behavioral recovery following brain damage (Karpiak, 1983; Li et al., 1986; Poplawsky, 1987; Sabel et al., 1984). In several cases, the biochemical and functional ameliorative effects of ganglioside have been associated with an increased survival of specific neuronal populations (Agnati et al., 1983; Commissiong and Toffano, 1986; Cuello et al., 1986; Sofroniew et al., 1986; Toffano et al., 1984b).

One interesting paradigm among the experimental models of neurochemical CNS lesions is that that develops following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP induces neurodegenerative changes in mammalian brain and clinical symptoms in humans that resemble Parkinson's disease, with loss of dopamine-containing nigrostriatal neurons (Heikkila et al., 1984). In this case also, treatment with GM1 results in elimination of the accompanying biochemical and behavioral deficits (Hadjiconstantinou et al., 1986; Weihmuller et al., 1988).

A relationship between in vivo effects of ganglioside and neuronotrophic factors—an interaction strongly supported from in vitro observations—may well exist. It is important to keep in mind that trophic factors also serve a functional role in the adult CNS where, for example, NGF affects forebrain cholinergic neurons (cf., Korsching, 1986). Trophic activities, including NGF, increase at the lesion site following damage (Nieto-Sampedro et al., 1983; Gasser et al., 1986). Intraventricular injections of NGF are found to prevent retrograde degeneration of septal cholinergic neurons (Hefti, 1986; Kromer, 1987; Williams et al., 1986). Also, ganglioside effects in vitro are obtained at concentrations (Leon et al., 1988) compatible with the ganglioside concentrations obtainable in the brain after its systemic administration in vivo (Ghidoni et al., 1986).

Recent findings support the hypothesis that monosialoganglioside can potentiate neuronotrophic factor effects in vivo. Cuello et al. (1989) reported that both NGF and GM1 prevented the biochemical and morphological changes accompanying lesions to rat basal forebrain neurons; NGF and GM1 acted synergistically to stimulate choline acetyltransferase activity in

the nucleus basalis magnocellularis following unilateral decortication and in cultured septal neurons (*see also* Di Patre et al., 1989). In a PNS model, Vantini et al. (1988) showed that exogenous GM1 facilitates the ability of NGF to antagonize vinblastine-induced sympathectomy in neonatal rats, as measured by evaluating noradrenergic innervation in the heart and spleen; GM1 itself was ineffective on vinblastine action.

The ability of GM1 to act in vivo appears to depend on the extent of the lesion applied (Gradkowska et al., 1986; Stephens et al., 1988; Toffano et al., 1984a), suggesting the need for a minimum level of endogenous neuronotrophic support. This idea is consistent with in vitro studies, where the facilitating action of GM1 is dependent upon a proper balance between permissive and retarding influences acting together with NGF or other neuronotrophic factors (Katoh-Semba et al., 1984; Skaper and Varon, 1985; Skaper et al., 1985).

This model implies that the potentiating effect of GM1 in vivo is related to an enhancement of neuronotrophic activity already present in the damaged tissue, and slowly increasing. Such trophic activity by itself would be inadequate after injury, especially during secondary neuronal damage, but would be made effective by the presence of GM1—in effect, allowing the neurons to surpass a critical "set point." The effects of ganglioside on CNS lesions may thus reflect a facilitation of neuronal action dependent on an injury-induced supply of trophic factor(s). In other words, gangliosides are more likely to act on cellular events that characterize a neuron's response to a neuronotrophic factor.

Brain Ischemia and Excitatory Amino Acid Neurotransmitter-Induced Neurodegeneration: Gangliosides as Neuroprotective Agents

The observed efficacy of monosialogangliosides like GM1 in improving neural behaviors following brain lesions has led several laboratories to explore the action of these molecules in paradigms where a brain insult is thought to result from the action of excitatory amino acid neurotransmitters. Under physiological circumstances, the excitatory amino acid glutamate (or related compounds) is released at the synaptic cleft, leading to postsynaptic action and mediation of key plastic responses such as long-term potentiation. Excessive release of glutamate under neuropathological situations, however, like cerebral ischemia, anoxia, or hypoglycemia may also be responsible for the consequent neuronal death (Rothman and Olney, 1986). Glutamate receptor antagonists are protective when applied to both animal models of ischemia, and to CNS neurons in vitro exposed to glutamate directly or to anoxia (Choi, 1988). These observations provide a strong basis for an excitotoxic hypothesis of neurotoxicity (Rothman and Olney, 1987).

Most strategies directed to neuroprotection have relied on glutamate, in particular, *N*-methyl-D-aspartate (NMDA) type antagonists. Unavoidable side effects associated with the use of any NMDA antagonists will be events directly attributable to altered synaptic transmission. Loss of normal NMDA receptor-mediated synaptic plasticity, perhaps affecting learning and memory (Collingridge and Bliss, 1987) could prove quite undesirable. Likewise, blocking Ca^{2+} channels could interfere with normal excitatory neurotransmission. However, a more novel approach to the problem of excitotoxin-induced neurodegeneration has made use of monosialogangliosides. GM1 (or its inner ester derivative) have been observed to protect the brain against various biochemical and functional deficits occurring in different experimental models of cerebral ischemia, either focal or global (Cahn et al., 1986; Karpiak et al., 1987,1988; Komatsumoto et al., 1988; Seren et al., 1989; Tanaka et al., 1986). Excitotoxin brain damage induced by ibotenic acid, a glutamate receptor agonist (Coyle, 1982) has

also been reported to be protected by GM1 administration (Mahadik et al., 1988).

Recent studies have shown that gangliosides, including monosialogangliosides, are able to reduce excitatory amino acid-related neurotoxicity in cultured cerebellar granule cells, under both normoxic (Favaron et al., 1988) and anoxic (Facci et al., 1989) conditions. Figure 6 illustrates the ability of GM1 and its inner ester derivative (siagoside) to protect against glutamate neurotoxicity. The following section will discuss some considerations for possible ganglioside mechanisms of action.

Molecular Mechanisms of Ganglioside Action: Implications in Membrane-Mediated Transfer of Information

Exogenous vs Endogenous Gangliosides

Ganglioside-initiated events, as well as the ganglioside-induced potentiation of neurotrophic factor effects on neuronal cells, very likely involve a modification of cell surface properties consequent to the stable insertion of the ganglioside. However, the molecular mechanism(s) underlying the biological effects of the ganglioside have yet to be identified. One important question is whether the exogenously inserted ganglioside molecules behave as do the endogenous ones.

Studies using artificial and natural membranes have shown that the concentration of gangliosides in a given membrane area is not static but depends on dynamic interactions among ganglioside polar head groups, divalent cations, and cell surface glycoproteins (Tettamanti et al., 1985). Gangliosides are able to spontaneously incorporate into the phospholipid structure of artificial membranes and display considerable lateral mobility (Sharon and Grant, 1978). Under the appropriate ionic environment, the gangliosides tend to concentrate into clusters, thereby affecting the curvature, local composition, and stability of the membranes (Maggio, 1985).

The examination of ganglioside organization in natural membranes presents a more difficult problem, since highly specific probes are generally not available. One notable exception is the B subunit of cholera toxin, which displays an almost absolute affinity for the ganglioside GM1 (Fishman, 1982). By using the B subunit together with fluorescently labeled cholera toxin antibodies, it has been possible to show that the distribution of endogenous GM1 molecules on the surface of lymphocytes at 4°C changes at 37°C to form a cap at one pole of the cells (Revesz and Greaves, 1975; Spiegel et al., 1984). This capping phenomenon is accompanied by cocapping of the cytoskeletal protein actin and is inhibited by the actin destabilizing drug cytochalasin B (Kellie et al., 1983), suggesting that GM1 may be associated with membrane proteins, and in turn linked to the cytoskeletal system. The B subunit has also been used to evaluate the role of membrane gangliosides in the regulation of fibroblast cell growth (Spiegel and Fishman, 1987).

Radioactively labeled GM1 is known to readily associate with cells in a temperature-, time-, and concentration-dependent manner (Callies et al., 1977; Facci et al., 1984; Radsak et al., 1982; Skaper et al., 1988). Other than a labile type of association with the cell surface, there occurs a stable, trypsin-resistant type of association, with the ganglioside intercalated into the outer layer of the membrane (Schwarzmann et al., 1983). Formation of caps has also been observed after addition of fluorescently labeled GM1 to lymphocytes treated with the cholera toxin B subunit (Spiegel et al., 1984). In addition, the stably associated GM1 is functionally and metabolically active (Fishman et al., 1983; Moss et al., 1976). This suggests that exogen-

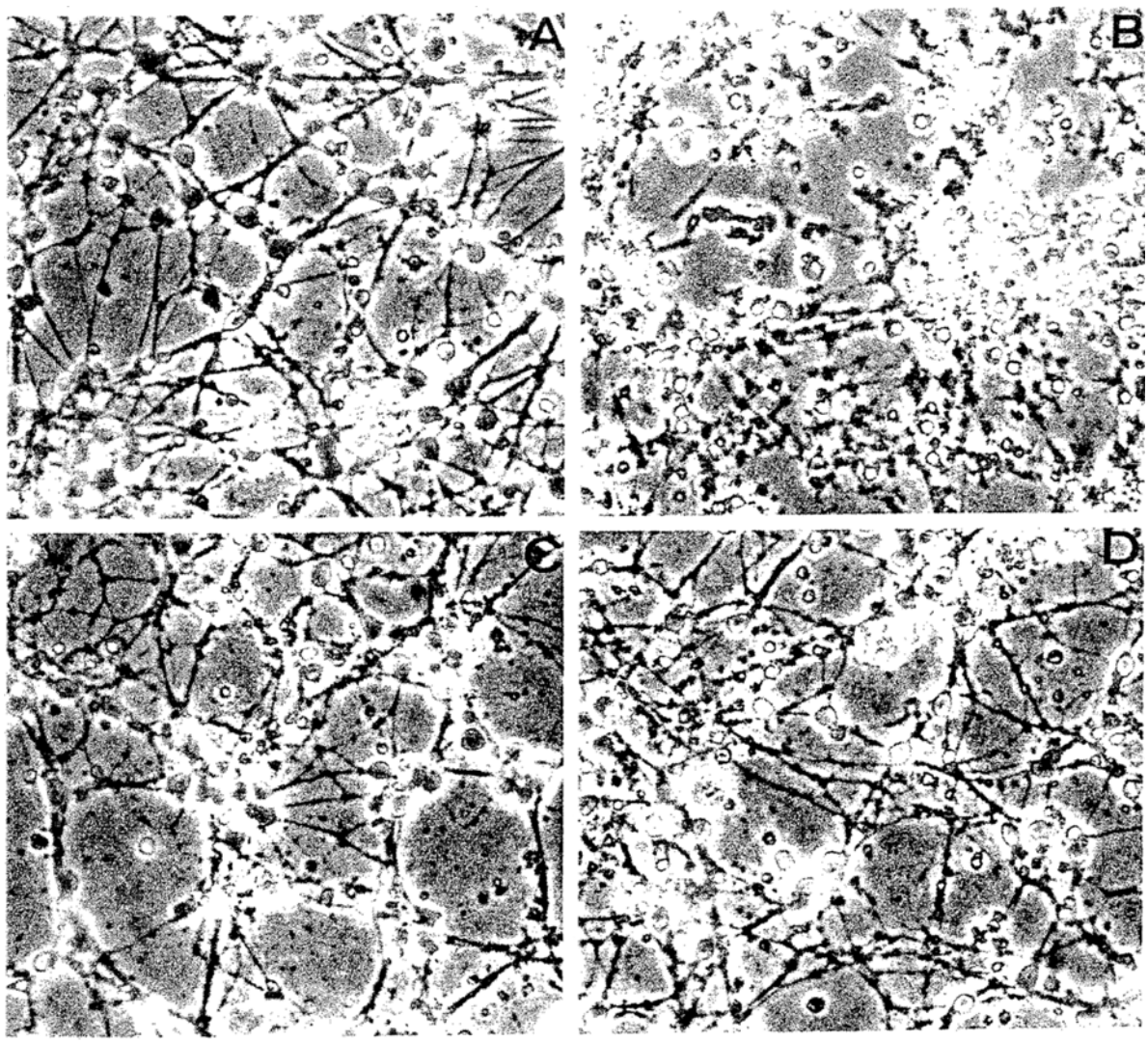

Fig. 6. Ganglioside GM1 and its inner ester derivative (Siagoside) prevent morphologic evidence of glutamate neurotoxicity. Granule cells were prepared from cerebella of 8-d-old rat pups (Gallo et al., 1982) and used after 12 d in vitro. Cells were treated with 100 μ*M* GM1 or Siagoside for 2 h, followed by washout with serum and a 3-h pulse of 500 μ*M* glutamate. Cultures were returned to their normal medium and photographed under phase microscopy 24 h later. Control (A), glutamate (B), glutamate plus GM1 (C), or siagoside (D).

ous gangliosides, once inserted into the plasma membrane, display a dynamic behavior similar to that of endogenous gangliosides. Good evidence for this also comes from the finding that treatment of astroglial cells (which contain endogenous GM1) with the B subunit markedly reduces DNA synthesis (Facci et al., 1988), an effect reproduced in C6 glioma cells (devoid of endogenous GM1) by insertion of exogenous GM1 or by treatment with neuraminidase to generate endogenous GM1 from poly-sialogangliosides (Skaper et al., 1988).

Ganglioside Modulation of Cell-Ligand Associated Events

Gangliosides seem to be involved in the molecular machinery responsible for cellular responses to external ligands. These situations are different from those where ganglioside interacts directly with the external ligand, e.g., in the action of cholera toxin B subunit. Rather, the ganglioside action is presumed to operate via modulation of protein complexes playing key roles in signal reception and transduction. Ganglioside-dependent modulation of membrane receptor function has been suggested by the modification of protein kinase activity of polypeptide growth factor receptors by gangliosides GM3 and GM1, but not by other types of glycolipids (Bremer et al., 1986; Hanai et al., 1988a,b). Furthermore, these observations correlate with the effects of exogenous addition of the gangliosides on mitogen-dependent cell growth stimulation (Bremer et al., 1986; Hanai et al., 1988a). Among other membrane-associated proteins known to be influenced by exogenous gangliosides are sodium channels (Carpenter et al., 1988; Spiegel et al., 1986) and enzymes, such as Na^+, K^+-ATPase (Esmann et al., 1988; Fass et al., 1987; Leon et al., 1981; Li et al., 1986; Vyskocil et al., 1985).

The activities of several protein kinases are also regulated by gangliosides. This ganglioside action may be particularly relevant, given that protein phosphorylation represents one of the most important post-translational modification systems in the regulation of biological processes. Most of the kinases affected are membrane-associated proteins, and have been studied in cell-free or whole-cell preparations; the ganglioside effects observed have been either inhibitory or stimulatory, depending upon the particular system. These proteins include two distinct enzymes from guinea pig brain (Chan, 1987a,1988), a Ca^{2+} ecto-kinase in GOTO neuroblastoma cells (Tsuji et al., 1985), a Ca^{2+}-dependent protein kinase from rat brain (Goldenring et al., 1985), Ca^{2+}/calmodulin-dependent protein kinase (Cimino et al., 1987), and protein kinase C (Cimino et al., 1987; Kreutter et al., 1987; Vaccarino et al., 1987). Although the exact physiological significance of ganglioside-responsive protein kinases is not known, it is possible that certain functions related to gangliosides in the nervous system are mediated through their activation or inhibition. One such case may be that of ganglioside-mediated protein phosphorylation in myelin (Chan, 1987b).

Ganglioside Implications in Plasma Membrane-Cytoskeletal Associated Events and Intracellular Responses

Exogenous, membrane-inserted gangliosides can interact not only with each other, but with cell surface glycoproteins (Felgner et al., 1983), suggesting that the inserted ganglioside may self-associate to form microdomains in the lipid bilayer. The formation of compositional domains with differing ganglioside content may cause local changes in membrane fluidity that can, in turn, influence processes taking place at the cell surface (e.g., receptors or kinases) as well as at the intramembrane level. The possibility that ganglioside clusters may be associated with intramembrane proteins connected with the cytoskeletal system suggests that gangliosides may be involved in the regulation of the metabolic response of the cell to external stimuli. An example is the implication of gangliosides in cell adhesion phenomena (Blackburn et al., 1986; Cheresh et al., 1986; Thompson et al., 1986).

Functional implications of gangliosides concerning a plasma membrane-cytoskeletal connection can also relate to the mechanism of genome expression. Either stimulatory or inhibitory effects can be observed, depending on the parameter under study. One example is that of the GM1-induced production of mRNA

for tubulin in a hybrid neuroblastoma cell line during differentiation (Rybak et al., 1983). GM1 is also reported to increase tubulin gene transcripts when administered following a CNS lesion (Yavin et al., 1987). A different case can be seen from studies using cultured astroglia. This CNS glia cell type undergoes a change in morphology in response to cyclic AMP- elevating agents that resembles, morphometrically, reactive astroglia in vivo. Treatment with GM1 prevents or reverses this morphological reaction (Skaper et al., 1986), independent of cyclic AMP (Facci et al., 1987). Such cyclic AMP-stimulating agents also increase levels of mRNA for Glial Fibrillary Acidic Protein (GFAP), a major cytoskeletal protein of astroglia; this nuclear response is reduced by GM1 (Skaper et al., unpublished observations).

A Working Model for the Action of Exogenous Gangliosides

Any model to define the molecular bases of ganglioside action must account for two aspects: 1. the relationship between gangliosides and their functional involvement, and 2. their ability to affect a wide variety of cellular events. The diversity of oligosaccharide chains of gangliosides imparts a high potential for specific binding to a variety of ligands. Attachment of this carbohydrate chain to a lipophilic ceramide moiety, on the other hand, provides for transmission of conformational changes to the membrane imparted by ligand binding. As the inserted ganglioside is now an integral part of the membrane, variability in ganglioside composition may be critical in conferring to the membrane microenvironment special properties in selecting membrane-associated proteins for modulation. Ganglioside interactions with external ligands and membrane components can influence membrane dynamics, leading to a local reorganization of membrane architecture. Such structural changes can be of importance in modulating the activity of membrane proteins, e.g., receptors, ion channels, and signal-transducing systems. Furthermore, the nature and extent of the endogenous ganglioside complement may influence the effect of the exogenous ganglioside (Hanai et al., 1988b). Figure 7 presents these concepts schematically.

The application of this scheme to exogenous ganglioside action on neuronal cell behaviors is consistent with currently available information. Ganglioside-mediated potentiation of neuronotrophic factor action can be viewed as operating via modulation of cell surface transduction events or enhancement of specific trophic factor-induced post-translational steps. The trophic protein initially binds to cell surface receptors, with the ensuing production of second messenger species. These messenger molecules, and/or the trophic factor complex itself, may eventually alter cellular processes at the nuclear level, the latter events expressing themselves as trophic effects. In the case of GM1 and NGF, PC12 cells treated with GM1 do not appear to alter the binding constant of their NGF receptors (Ferrari et al., 1983). The entire sequence of events in NGF interaction with its target neuron receptors—binding, sequestration, and internalization—has not yet been fully explored, however, under GM1 action. One post-translational event, namely, NGF stimulation of tyrosine hydroxylase phosphorylation, is reported to be potentiated by GM1 (Hilbush and Levine, 1988) in PC12 cells. Phosphorylation of this enzyme, the rate-limiting step in catecholamine neurotransmitter synthesis, is necessary for its activity.

The action of gangliosides in modulating excitatory amino acid neurotoxicity can also fit within this model. Glutamate treatment of granule cells is observed to provoke a translocation of protein kinase C from cytosol to membrane (Vaccarino et al., 1987), a process that depends on extracellular Ca^{2+}. A pre-exposure of the cells to ganglioside diminishes this translocation, without affecting glutamate

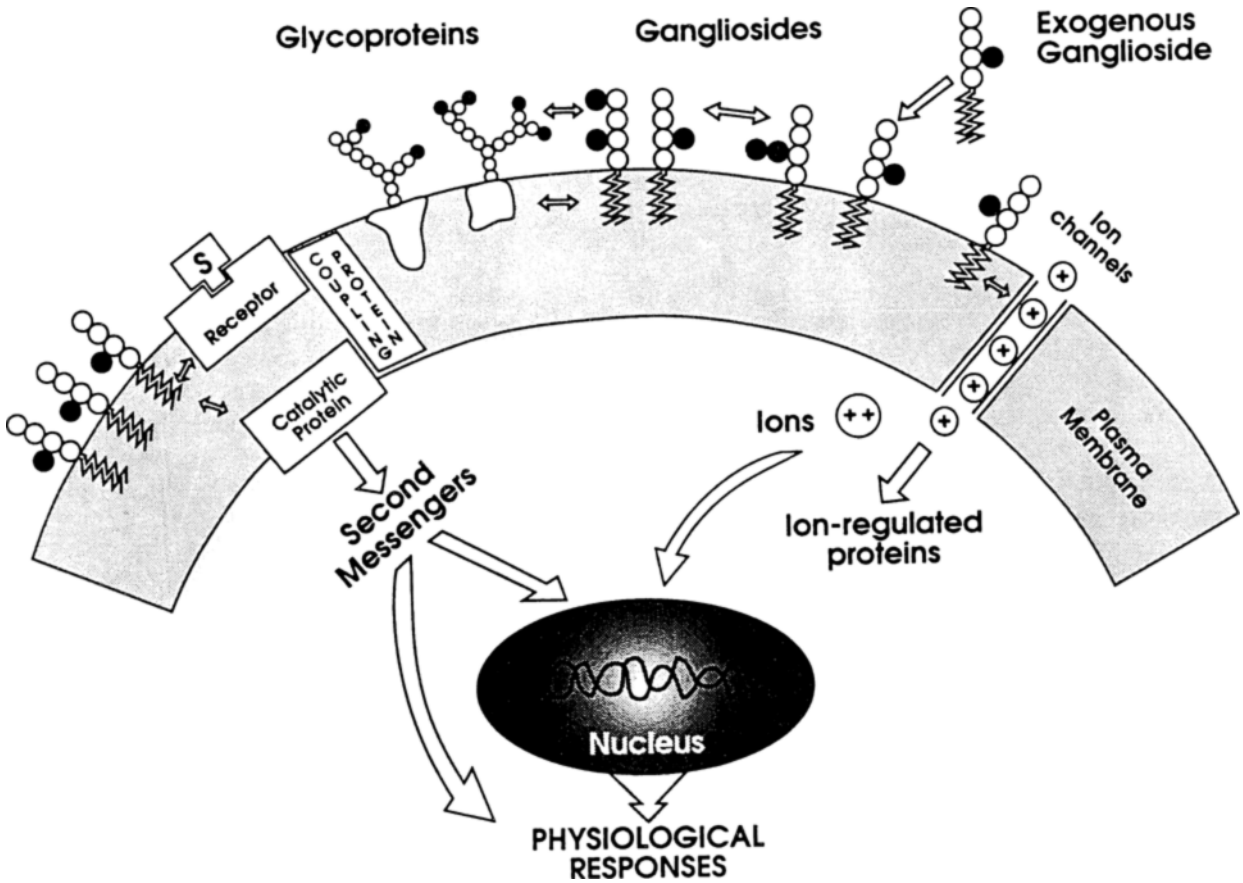

Fig. 7. A proposed model for exogenous ganglioside action as mediator of information transfer across the plasma membrane. The inserted gangliosides can be envisaged as: modulating extracellular matrix proteins or interacting with external ligands; modulating functional membrane proteins; or modulating intracellular response mechanisms (e.g., genome expression).

binding to its receptor (Vaccarino et al., 1987) or glutamate-triggered intracellular signals, like c-fos induction, opening of receptor gated Ca^{2+} channels or phosphoinositide hydrolysis (Favaron et al., 1988). Thus, gangliosides seem to influence the neuronal response to an extracellular cue (glutamate) not at the level of receptor-activated signal transduction, but rather at the level of secondary cellular responses. In contrast to antagonists directed to glutamate recognition sites, the action of gangliosides would not be expected to adversely impact upon normal neuroplastic behaviors.

Perspectives for the Future

Understanding the mechanisms underlying neuronal plasticity is one of the major tasks facing neurobiological research today. The central nervous system is no longer considered to be a static and structurally irreparable unit. Experimental evidence shows that even the mature CNS carries the potential for structural reorganization and the resulting functional recovery following brain damage. This intrinsic neuroplasticity, in response to external noxious stimuli, may be mediated though endogen-

ous factors. At the same time, microenvironmental cues may also carry restrictive signals directed to neurons. The ability to alter the responses of neuronal cells to such extrinsic influences will formulate a powerful means for modulating the neuroplastic behaviors of these cells—a critical consideration for promoting regeneration and repair processes in the brain. As we have discussed in this article, gangliosides—a class of naturally occurring glycosphingolipids—have been shown in many studies to have the capacity to reduce and even reverse the consequences of damage to the nervous system induced by toxic, traumatic, ischemic, or metabolic causes.

Disruption of this finely tuned balance of extracellular influences by axotomy or disruption of the blood supply are probably the two major causes of neuronal death in the CNS; in some instances, both sequelae may be operative. Functional repair/recovery of the damaged CNS will require, at least in part, interventions that are directed at manipulating the brain's own plastic reactions. The evidence regarding the effectiveness of monosialoganglioside administration on the outcome following different paradigms of experimentally-induced acute brain damage has led to studies on the potential use of GM1 in the pharmacotherapy following acute brain injury, especially stroke, in humans. Preliminary clinical studies have shown that GM1 has a favorable effect on the rehabilitation of patients following ischemia or cerebral hemorrhage (Argentino et al., 1989; Bassi et al., 1984,1986; Battistin et al., 1985). Ganglioside GM1 has also been reported to have a favorable effect on the recovery of neurological and neuropsychological deficits during the rehabilitation of patients following closed traumatic head injury (Hörmann, 1988). Furthermore, gangliosides have found application in the treatment of a number of peripheral neuropathies (cf., Massarotti, 1986).

The information discussed here supports the potential pharmacological action of gangliosides in improving the recovery of nerve cell distress or impairment due to insufficiencies of neuronotrophic influences or excesses of neuronotoxic activities. These two aspects are not necessarily mutually exclusive: conceivably, neuronal cell viability and function will ultimately depend on the balance between these two types of extracellular signals. Clearly, much work remains to be done. It is hoped that this article will provide a stimulus for further studies to define the cellular and molecular mechanisms underlying the ganglioside effects in vivo, as well as their action in therapeutic settings.

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