0734-600X/86/0503-0309$04.20

Gangliosides in the Nervous System During Development and Regeneration

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Received April 4, 1986; Accepted June 9, 1986

ABSTRACT

Gangliosides are present in nervous tissues of echinoderms and chordates, but the amounts and patterns differ widely. There are changes in the ganglioside contents of nervous tissues during development in most animals studied. To a large extent, regional differences and changes with development and degeneration in ganglioside composition reflect changing and different proportions of cellular types and subcellular organelles within the tissue. G_{M1} and G_{M4} are enriched in myelin; G_{D1a} may be a marker for dendritic arborization. During regeneration of fish optic nerve and rat sciatic nerve there is an increased amount of ganglioside proximal to the regenerating axon tips, which may largely be a result of accumulation. This could provide a relatively large reservoir of ganglioside to become incorporated into the sprouting axolemma. Gangliosides added exogenously to growth medium can induce neuritogenesis of several types of neurons. The mechanisms of this action are unknown but may be related to nerve growth factor, microskeletal organization, membrane fluidity, and other factors. Gangliosides injected into young animals affect brain development, but further studies are required to determine these effects more specifically. Ganglioside administration increases the number of sprouts in regenerating peripheral nerves, but does not seem to accelerate axonal elongation. Parenterally administered gangliosides alter the recovery of brain tissue from a variety of types of lesions, and clinical trials are in progress to determine if they are of benefit in human neurological disorders. The biochemical mechanisms of these in vivo ganglioside effects are

poorly understood, but may involve modulation of several enzyme systems as well as other properties of neural membranes, such as fluidity. It is possible that gangliosides may play similar roles and operate through some of the same mechanisms in developing and regenerating nervous tissues.

Index Entries: Gangliosides, development and regeneration of; gangliosides, and nervous tissue; ochinoderms; chordates regeneration, of nervous tissue; gangliosides, and development, gangliosides in; gangliosides, nervous tissue of.

CHANGES IN GANGLIOSIDES DURING NEURAL DEVELOPMENT

Gangliosides are present only in echinoderms and chordates, but within these phyla there are marked differences among animal species, and even strains, in the amounts and patterns of brain gangliosides. Complex patterns containing polysialogangliosides have been found in elasmobranchs, teleosts, amphibians, and reptiles (Irwin, 1984). In general, mammals and birds have fewer ganglioside fractions and less of the very polar gangliosides, with fewer or more sialic acids than phylogenetically lower animals (Rahmann and Hilbig, 1983; Hilbig, 1984; Hilbig et al., 1981). The combined evidence that gangliosides may be involved in neuritogenesis and that animals that have greater neural regenerative capacities have more polysialogangliosides in brain led to the suggestion that the more complex gangliosides may be involved in CNS regeneration of fish and amphibians. Tadpoles are capable of regenerating central nervous tissues to a certain degree, but adult frogs are not (Michel and Reier, 1979; Singer, 1954). We thought that this might be related to the brain ganglioside patterns, which would be expected to change during metamorphosis. Gangliosides were extracted from brains of *Rana catesbiana* during three stages of metamorphosis (early premetamorphic, late premetamorphic, and climax) and from adult bullfrogs. Using one dimensional high-performance thin-layer chromatography (HPTLC), nine gangliosides were identified and the relative proportions of these determined using scanning densitometry (Yates et al., 1985b). It was surprising that during this period of tremendous growth and differentiation there was no change in the pattern of brain gangliosides. This suggests either that gangliosides are not involved in CNS regeneration of these tadpoles and/or that during metamorphosis some other aspect of the regenerative mechanism is lost or suppressed.

Variations in ganglioside contents among different brain regions are well documented. For some gangliosides, these differences are known to reflect variable proportions of different cell types and subcellular structures among these regions. For example, cerebral white matter has a higher proportion of G_{M1} and G_{M4} than cerebral cortex because these two

gangliosides are relatively enriched in myelin (Suzuki, 1970; Yu and Iqbal, 1979). However, the bases for some other regional differences, such as the higher proportions of G_{T1b} in cerebellum than cerebrum, are not known (Vanier et al., 1971).

Considering the phylogenetic, regional, and subcellular differences in the composition of adult brain gangliosides, it is not surprising that differences also occur in their patterns of change with development. In both the rat and human cerebral cortex there is an approximately threefold increase in ganglioside concentration during postnatal development (Vanier et al., 1971). The major increase in the rat occurs between birth and 17 d of age; in the human, this occurs between the 15 wk of gestation and 6 mo of postnatal life. These changes in patterns occur in three phases. The first is the time when there is multiplication of neurons and glia, during which G_{M1} and G_{T1b} are the major species. In the second phase, the multiplication of glia and small neurons continues, as well as dendritic and axonal growth and synaptogenesis. Concurrently, there is a proportional increase in G_{D1a} and a decrease in G_{T1b} and G_{M1}. In the third phase, neuronal process formation and synaptogenesis continue and myelination occurs. During this time, G_{D1a} stabilizes as the major ganglioside. Different changes in ganglioside patterns occur in human cerebral white matter. On a fresh weight basis, total ganglioside increases slightly between birth and 4 mo of age, after which it decreases to the adult level by 10 mo. At birth, the ganglioside pattern of presumptive white matter is similar to that of cerebral cortex (G_{M1} and G_{D1a} are the major species). The proportion of G_{M1} increases to the adult level by 1 yr of age, whereas G_{D1a} continuously decreases through 30 yr. The increased amount of G_{M1} is caused by myelination. These observations have been extended using mouse and rat brain (Hilbig et al., 1982; Yavin and Yavin, 1979; Irwin et al., 1980; Irwin and Irwin, 1979). From gestational d 10 until birth, the proportions of G_{Q1b}, G_{T1b}, and G_{D1a} increase at the expense of G_{M3} (mouse) and G_{D3} (mouse and rat). During the first postnatal week, G_{M3} and G_{D3} continue to decrease as G_{D1a} increases. Concomitant with myelination, G_{M1} increases and G_{T1b} and G_{Q1b} decrease. These changes in ganglioside profiles occur with a generally similar time course in cells cultered from 16-d-old rat brains (Yavin and Yavin, 1979). In cerebra and optic lobes of developing chick, alterations in ganglioside patterns also parallel structural changes (Rosner, 1980). G_{D3} is the predominant ganglioside during the period of neuroepithelial cell proliferation, but increases in G_{T1b} and G_{D1a} occur during neurite growth and synapse formation. Similar changes also occur in primary cultures of chick brain neurons (Dreyfus et al., 1980), in which G_{D3} is the major ganglioside during the period of cell division, but progressively decreases during the first week of culture. G_{D1a} increases between 3 and 7 d of culture and becomes the major one between 5 and 7 d, when neuronal maturation and synaptogenesis is occurring. Consistent with this are the results of ganglioside analyses on subcellular fractions of rat brains at 21 and 81 d

of age (Yusuf and Dickerson, 1978). During this time cerebral fresh weight increased 22%, whereas total ganglioside content increased 52%. G_{D1a} accounted for 39% of this increase. A 71% of the increase in cerebral G_{D1a} occurred in the microsomes and 15% in the synaptosomes. From these results the authors concluded that in rat cerebrum, G_{D1a} may be a marker for dendritic arborization. Thus, there is a generally consistent finding that G_{D1a} increases concomitant with neuronal differentiation. In cultures of chick glial cells, G_{M3} and G_{D3} are the major gangliosides, and there is very little polysialoganglioside even after 25 d of culture (Dreyfus et al., 1980).

Changes with age in the ganglioside composition of myelin occur in mouse (Yu and Yen, 1975) and rat (Suzuki et al., 1967), but not chick (Cochran et al., 1983). In these two mammals, the proportions of G_{M4} and G_{M1} in CNS myelin progressively increase with age. The role of gangliosides in myelin is not known, but they may be of importance in intermolecular interactions with myelin basic protein (Cochran et al., 1983).

Differences in changes of ganglioside patterns with age have been found among different brain regions, such as cerebellum and cerebrum (Vanier et al., 1971), and even in subregions, such as within the hippocampus (Irwin and Irwin, 1979, 1982). However, these correlate with the same general phases of structural maturation discussed above.

Two dimensional thin-layer chromatography (2D-TLC) has revealed considerable heterogeneity within many of the bands resolved by one dimensional chromatography. Using 2D-TLC Chigorno et al. (1984), have demonstrated developmental changes in the alkali-labile gangliosides. The proportion of alkali-labile gangliosides in rabbit cerebellum increases from 5% at birth to 17% at 6 mo of age. In cerebrum there were lower amounts of alkali-labile gangliosides, and less marked changes occurred with development. The complexity of brain gangliosides recently recognized by techniques such as these and HPLC (Kadowaki et al., 1984) have made the problem of correlating specific gangliosides with cell types, subcellular fractions, or developmental processes much more difficult.

Hitzemann and Harris (1984) studied the effects of age on the lipid composition and fluidity (order) of rat synaptic membranes. Between 3 and 120 d of age, fluidity of the hydrophobic core significantly decreased. A similar change was seen in liposomes prepared from synaptic membrane lipids. Synaptic membrane surface fluidity did not change with development as much as the membrane interior, but surface fluidity of liposomes with total lipid extracts prepared from synaptic membrane did increase with age. Gangliosides had a significant effect on this membrane surface fluidity. Ledeen et al. (1985) have recently reported the lipid composition of growth cones isolated from embryonic rat brain. Compared with adult synaptic plasma membranes, the ratio of ganglioside to protein is higher, but ganglioside to total phospholipid is lower. It would be

of considerable interest to know the growth-cone-membrane surface and interior fluidities and to determine the contributions of the gangliosides to these properties. It is possible that gangliosides could be involved in several important developmental phenomena, such as neurite initiation, branching, and synaptogenesis.

Gangliosides in peripheral nerve have also been studied during development. The concentration of total ganglioside in rabbit sciatic nerve (SN) increases between birth and 2 wk of age, but then decreases to adult levels by 8 wk (Yates and Wherrett, 1974). Between birth and 1 yr of age, no change in ganglioside pattern was found, but these studies should be repeated using HPTLC and HPLC, which give better resolution of ganglioside species. Rat peripheral nerve has a different ganglioside composition than that of rabbit or chicken, and major developmental changes are seen in the ganglioside profiles of rat nerve (Chou et al., 1982). At birth, G_{M3} is the major ganglioside, the proportion of which decreases considerably within the first 3 wk of life. L_{M1}, a glucosamine-containing ganglioside, is a relatively minor component at birth, but increases to become the major ganglioside in adult nerve. L_{M1} is probably located in peripheral nerve myelin.

GANGLIOSIDES IN DEGENERATING AND REGENERATING NERVOUS TISSUES

The effects of trauma on the synthesis and composition of gangliosides has received relatively little attention. In one study, optic nerves of goldfish were crushed, and after several time intervals the eyes were injected with a radiolabeled precursor of ganglioside synthesis. During regeneration, there was an eightfold increase in the amount of radiolabeled ganglioside within the visual pathway distal to the crush site, but proximal to the optic tectum (Sbaschnig-Agler et al., 1984). This increase could have been a result of increased ganglioside synthesis by the retinal neurons with axons projecting into the optic nerve and/or to the accumulation of ganglioside proximal to the axon tips. In either case, it provides an increased amount of ganglioside available to be incorporated into the growing axonal tip. This could increase the concentration of ganglioside within the terminal axolemma to levels higher than elsewhere in the axon and lead to lipid compositions and membrane properties similar to those of embryonic growth cones. Therefore, some of the mechanisms regulating the ganglioside contents of the growing tips of normal embryonic or traumatized adult axolemma may be similar.

During Wallerian degeneration of transected rabbit SN, the total ganglioside content increases 64% by 3 wk after trauma (Yates and Thompson, 1978). During this same time, gangliosides that comigrate with G_{M2}, G_{M3} and G_{D3} increase, and those comigratory with G_{T1b}, G_{D1b}, and G_{D1a} decrease (Yates and Thompson, 1978; unpublished observa-

tions). The interpretation of these results is that G_{T1b}, G_{D1b}, and G_{D1a} are mainly located in the axons, whereas G_{M3}, G_{M2}, and G_{D3} are mainly in Schwann cells.

We also studied the effects of nerve transection on the synthesis and accumulation of gangliosides in the proximal stump of transected rabbit SN (Yates et al., 1985a). At several different times following transection of the left SN, a precursor of ganglioside synthesis, [^{3}H]glucosamine, was injected into both L-7 dorsal root ganglia (DRG). At the time of injection, the right SN was ligited at the same level that the left nerve had been transected to control for accumulation of ganglioside proximal to the lesion site. Two days later the animals were killed, the nerves removed, and the gangliosides purified from both the proximal stump of the nerve [lumbosacral trunk (LST)] and the L-7 DRG. Expressing the data for the radioactivity in gangliosides from the LST as a percent of total nerve radioactivity, there was no difference between control and transected nerves. This suggested that there was no change in the rate of synthesis of gangliosides in response to nerve transection. However, the control nerves had been ligated to control for accumulation, so it is possible that this degree of trauma could have provided sufficient stimulus to alter ganglioside synthesis.

A second series of experiments was conducted using rats. In these studies, the left SN was crushed and both L-5 DRG injected with [^{3}H] glucosamine at different time intervals following crush. The right nerves were not ligated. Two days later the animals were killed and the gangliosides purified from the DRG, LST, and SN. Data were expressed as total amount of radioactivity in gangliosides of each of these nerve segments. At 48 h after trauma, the crushed LST had three times as much radioactivity in the gangliosides as did the control LST. This difference progressively decreased so that after 2 wk following crush, there was no difference between experimental and control values. For the first 3 d following crush, the amounts of radioactivity in gangliosides of crushed SN were either similar to or lower than control SN. However, 4 d after crush, when the regenerating nerve sprouts would have entered the SN, the levels of radiolabeled gangliosides in crushed SN were more than twice the control values. By 2 wk, the values for crushed and control SN were again equal. No significant differences were seen in amounts of ganglioside radioactivity between the left and right DRG. We interpret these results to mean that the amount of ganglioside synthesized by neurons in the DRG does not change following crush injuries to their axons. However, there is an accumulation of transported gangliosides proximal to the crush site. This seems to be a similar phenomenon to what has recently been reported for crushed optic nerve of goldfish (Sbaschnig-Agler et al., 1984). As discussed above, the increased amounts of accumulated ganglioside proximal to the axonal tips could serve as a reservoir to be incorporated into the growing tip, where it could promote axonal sprouting. It is also possible that a considerable amount of the recently

synthesized ganglioside is in the growth cone itself. If so, it might be useful as a growth-cone marker.

EFFECTS OF EXOGENOUSLY ADMINISTERED GANGLIOSIDES

Observations In Vitro

The addition of gangliosides to culture medium has a variety of biological effects that differ among cell types. Exogenously added gangliosides inhibit the growth of several types of neural and extraneural cells, and we found that ganglioside supplementation of growth medium inhibited the growth of normal human fetal brain, but not glioma cells (Icard-Liepkalns et al., 1982). Human beta-interferon inhibited the growth of the glioma, but not fetal brain cells (Yates et al, 1985c). Gangliosides bind to and neutralize beta-interferon, and it had been postulated that ganglioside may be at least part of the interferon receptor (Kuwata et al., 1978; Vengris et al., 1976). One general model for the action of gangliosides is that exogenously added ganglioside becomes inserted into the cell membrane, where it functions as a receptor for growth and/or survival factors (Morgan and Siefert, 1979). Therefore, we preincubated normal fetal brain and glioma cells with gangliosides before adding interferon (Yates et al., 1985c). However, ganglioside preincubation had no effect on the growth inhibiting effects of interferon for either cell line. Therefore, we concluded that ganglioside is probably not part of the human beta-interferon receptor in these cell lines.

A variety of cultured cells of neuronal origin demonstrate markedly enhanced neurite formation in response to exogenous gangliosides. These include neuroblastoma (Byrne et al., 1983; Facci et al., 1984; Roisen et al., 1982; Tsuji et al., 1983), pheochromocytoma (Ferrari et al., 1983), and DRG (Leon et al., 1984) and fetal brain of chick (Dreyfus et al., 1984) and rat (Savettieri et al., 1983/84). Ganglioside treatment of neuroblastoma cells increases the organization of microfilaments, especially in the growth cones and distal portion of the neurite, but has little effect on microtubules (Spero and Roisen, 1984). Nevertheless, gangliosides added to cultures of a neuroblastoma hybrid (SB21B1) stimulate neurite extension, which is accompanied by an increased expression of mRNA for tubulin but not actin (Rybak et al., 1983). Perhaps gangliosides can affect the microfilament and microtubulin systems differently in different cell types and under different growth conditions. The relationship between sprouting of chick DRG cells induced by NGF and that caused by G_{M1} was studied, estimating the degree of neurite formation by means of an ELISA assay for neurofilament protein (Doherty et al., 1985). G_{M1} alone did not support neuronal survival or induce neurite formation the way that NGF could. However, G_{M1} did augment neurite maturation in

the presence of NGF, and alone it enhanced neurofilament protein expression if NGF had been present in culture media for the immediately preceding 48 h. These results suggest that G_{M1} does not affect the cells simply by augmenting the amount of NGF bound to them, and it was concluded that G_{M1} was exerting its effect at the signal execution, not the signal reception, level. As discussed below, other investigators have obtained evidence that G_{M1} can function as a low-affinity receptor for NGF (Schwartz and Spirman, 1982).

There has been concern that the biological responses observed following the addition of ganglioside preparations to cell cultures may not be caused by gangliosides, but by contaminating peptides or proteins. Byrne et al. (1983) conducted an extensive study in which several different gangliosides were highly purified and then tested for their ability to induce sprouting in neuro-2A neuroblastoma cells. Some crude ganglioside preparations failed to induce neurites. However, following removal of peptides from these preparations they did demonstrate neuritogenic activity. All 11 specific gangliosides tested were active, although G_{M4} was slightly less so. Several neutral glycolipids, sulfatide, and free sialic acids were all inactive. It was concluded that gangliosides are neuritogenic agents, but care must be taken to remove inhibitory peptides from ganglioside preparations before testing them for biological activity. It should also be noted that even these extensively purified preparations still contained very small amounts of contaminating peptides.

The available evidence is convincing that ganglioside preparations have neuritogenic activity in vitro. However, this does not necessarily mean that the normal mechanisms of neurite formation involve gangliosides. Evidence concerning this was obtained using antiganglioside antibodies. Under appropriate growth conditions, neurons of goldfish retinal explants demonstrate neuritic outgrowth. However, this outgrowth is inhibited by antibodies directed against gangliosides isolated from bovine white matter (Spirman et al., 1982). Anti-G_{M1} antibodies also block sprouting of cultured chick DRG induced by NGF (Schwartz and Spirman, 1982). Thus, it seems that gangliosides, or substances that cross-react with them, are involved in this neuritogenic process. Schwartz and Spirman (1982) suggest that G_{M1} acts as a low-affinity NGF receptor that is blocked by the antibody.

Neurons cultured from brains of rat embryos extend neuritic processes, beginning within the first 24 h of culture (Yavin et al., 1984). Incorporation of radiolabeled glucosamine into glycolipids of these cells was much greater after 8 d than after 1 d of culture, and tunicamycin blocked labeling of glycolipids at 8 d more than at 1 d. Treating 8-d-old cultures with tunicamycin caused only slight changes in the ganglioside patterns, as determined by resorcinol staining, but major changes in the patterns of radiolabeling of gangliosides. Tunicamycin had less of an effect on glycolipid labeling at earlier culture times, when neurite outgrowth occurs most vigorously. From these results, the authors concluded that

glycolipid synthesis is not critical for neurite outgrowth. However, it is possible that the small amounts of glycolipids present during the early phases of culture are sufficient for the neuritogenic process. The apparent discrepancy between these studies and those using antiganglioside antibodies is interesting and deserves further investigation.

Observations In Vivo

General

Within the past decade, considerable interest has developed in the possibility that parenterally administered gangliosides may promote functional recovery of damaged nervous tissues. A wide range of ganglioside effects have been reported to occur in both central and peripheral nervous systems subjected to a variety of different forms of trauma. There are several interesting aspects common to these studies. One concerns the amounts of administered ganglioside that reach the tissues of interest. Tettamanti and coworkers (1981) have studied the pharmacokinetics and tissue distribution of injected tritium-labeled G_{M1} in the mouse. G_{M1} circulates in blood when injected by the intravenous (iv), intramuscular (im), and subcutaneous (sc) routes. The peak amounts measured occurred at 1 h for iv and im, but between 4 and 5 h for sc injections. In serum, G_{M1} micelles bind to albumin molecules in a 1:1 ratio, and these complexes can form dimers (Tomasi et al., 1980). Intravenously administered G_{M1} is taken up by brain, but begins to be metabolized within the first hour. Radioactivity present in the brain lipid fraction (most of which is G_{M1}) peaks within the first 2 h, and volatile radioactivity (most of which is probably in water) rises to a higher peak at 4 h after injection. At both 1 and 4 h after injection over 80% of the radioactivity in brain is in the soluble fraction and only 10% recovered in particulate fractions. Within 4 d of iv administration of [^{3}H]G_{M1}, approximately 38% of the radioactivity is excreted in urine. Of the remainder, some may be exhaled through lung or eliminated in feces via the biliary system. Gorio et al. (1980) found that after iv administration of [^{3}H]G_{M1}, the nonvolatile radioactivity in mouse SN peaked between 3 and 4 h after injection.

The doses of administered ganglioside have varied widely from 40 mg/patient/d in clinical studies (Bradley, 1984) to 50 mg/kg in animals (Gorio et al., 1980). Investigations with animals commonly use the higher doses. Calculations based on the data of Tettamanti et al. (1981) indicate that at 4 h after injection the radioactivity in brain derived from administered [^{3}H]G_{M1} could reach levels equivalent to approximately 40 μg ganglioside NeuAc/g brain weight or 4% of total mouse brain ganglioside. G_{M1} only accounts for 5–12% of mouse brain ganglioside (Seyfried et al., 1978) and much of this is in myelin. Thus, it seems possible that systemic administration of G_{M1} might cause significant, although transient, elevations of G_{M1} within some brain compartments. Calculations based on the data of Gorio et al. (1980) lead to a similar conclusion that

systemically administered G_{M1} could elevate the ganglioside content of the rat LST by about 4%. These calculations assume that the pharmacokinetics are linear between 0.5 and 50 mg/kg, and are based on an administered dose of 50 mg/kg. It should be emphasized that not all of the radioactivity in the brain or nerve was present as ganglioside at 4 h, but the results of these calculations indicate how much G_{M1} had reached these tissues within that time period.

Results of toxicological studies of ganglioside mixtures containing G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b} prepared from bovine brain have been reported (Heywood et al., 1983). The LD_{50} varied between 1.1 and 4.1 g/kg, and no target organ toxicity could be defined. The mixture had no teratogenic effect or adverse consequence on reproduction. Pups from mothers treated during pregnancy were apparently normal through to weaning, although maternal weight gain was slightly retarded at a dose of 100 mg/kg/d. Long-term effects of such treatment were not studied. Passive cutaneous anaphylaxis tests in rat, beagle, and guinea pig were negative, as were the histamine release from isolated lung and the bronchial resistance test in the guinea pig. Gangliosides are antigenic, but usually require administration with an adjuvant to induce antibody production. Nevertheless, it is important to be cognizant of the fact that antiganglioside antibodies may be produced in response to exogenously administered gangliosides, especially considering that most sources of injected gangliosides are prepared from animals of different species. Although several of the mammalian brain gangliosides are similar in commonly used experimental animals and man, documented species differences do exist. Differences also exist in ganglioside compositions of brain and peripheral nerve. It will be important to determine if the biological activity of some gangliosides is organ or species specific. The internal ester of G_{M1} (AGF2) is more active at low doses than G_{M1} in its effect on striatal tyrosine hydroxylase activity. The half life of AGF2 in serum is slightly longer and its distribution volume a little larger than G_{M1}. Thus, there is reason to believe that small structural changes in gangliosides may alter their biological activities.

Peripheral Nervous System

Ceccarelli et al. (1976) first observed in cats that parenteral administration of gangliosides could promote functional regeneration of peripheral nerve. This has been confirmed in several different systems employing a variety of methods (Kalia and DiPalma, 1982; Mengs et al., 1984; Norido et al., 1981; Sebille, 1984). Local application of gangliosides to crushed rat SN increased the number of regenerating axons, but did not accelerate axonal elongation (Sparrow and Grafstein, 1982). The latter observation has been independently confirmed (Verghese et al., 1982). Local and parenterally administered gangliosides slightly increased the number of axons in the regenerating limb bud of newts (Maier and Singer, 1984). This hyperinnervation was felt to be responsi-

ble for the accelerated limb regeneration seen in animals that received gangliosides. Parenterally administered gangliosides also increase the rate of motor nerve sprouting and reestablishment of functional neuromuscular junctions following nerve crush (Caccia et al., 1979; Gorio et al., 1980; Kleinebeckel, 1982). Ganglioside administration to patients has resulted in both subjective and electrophysiological improvements in patients with diabetic neuropathy (Crepoldi et al., 1983) and alcoholic neuropathy (Massoroti, 1983) and return to normal fiber spectra in SN of db/db diabetic mice (Norido et al., 1984). However, therapeutic trials of gangliosides have had no beneficial effect on humans with motor neuron disease (Harrington et al., 1984) or an animal model of this disorder, the Wobbler mouse (Lange et al., 1983). Overall, it appears that parenterally administered gangliosides promotes sprouting of traumatized peripheral nerve, but the clinical usefulness of this has yet to be definitely determined.

Central Nervous System

Considerable evidence has recently accumulated that indicates that parenterally administered gangliosides facilitate functional recovery of several brain systems from traumatic lesions. The mechanisms for this are still unclear. However, they may involve enhancement of neuronal survival (Norido et al., 1983; Sabel et al., 1984a,b) and promotion of structural reorganization, such as sprouting and neosynaptogenesis (Sabel et al., 1984a,b). Sparrow et al. (1984) found that antiganglioside antibodies inhibit regeneration of crushed optic nerve in goldfish, which strongly supports the idea that gangliosides are involved in axonal regeneration of this system. Evidence for a beneficial effect of gangliosides on CNS regeneration include: reduction of apomorphine-induced rotational behavior following nigrostriatal trauma (Agnati et al., 1983a,b); improvement in spatial reversal performance in animals with bilateral lesions in caudate nuclei (Sabel et al., 1984a,b); enhanced learned alternation in rats with unilateral entorhinal lesions (Karpiak, 1983); more normal open-field behavior in rats with bilateral entorhinal lesions (Fass and Ramirez, 1984); recovery of choline acetyltransferase and acetylcholinesterase activities in rat hippocampus following septal lesions (Wojcik et al., 1982); diminution of dopamine receptor supersensitivity following haloperidol administration to rats (Agnati et al., 1983a,b); and increased dendritic length of dopaminergic neurons of the zona reticulata, elevation of homovanillic acid content and tyrosine hydroxylase activity in the striatum, and reduction of dopamine cell degeneration in the substantia nigra of rats with ipsilateral lesions of nigrostriatal pathways (Toffano et al., 1983a,b; 1984a,b). Grafstein et al. (1983) found a bimodal effect of gangliosides on the startle reaction in goldfish following a crush lesion of the optic nerves. At lower doses of gangliosides, the recovery time was decreased, but at higher concentrations it was increased. The reasons for this are obscure, but may be related to the different ways gangliosides

bind to membranes at different concentrations. Many of these alterations were thought to be caused by gangliosides promoting the sprouting of dendrites that provide trophic support to other neurons through new dendro-dendritic interactions (Agnati et al., 1983a,b).

Very little information is available on the effects of exogenously administered gangliosides on the developing nervous system. In the only published study on this topic, neonatal rats were administered gangliosides between 5 and 15 d of postnatal life (Karpiak et al., 1984). Those that received mixtures of G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b}, or only G_{T1b}, G_{M1}, or G_{D1b}, performed better in a multidirectional avoidance paradigm when tested between 9 and 28 d of age. Acetylcholinesterase activities in cerebral cortex were also elevated in rats demonstrating improved behavioral performance compared to saline-treated controls. The authors conclude from this that administration of some gangliosides accelerate CNS maturation. However, it should be emphasized that these studies were very short term and the animals quite young when tested. Preliminary data from studies we performed show that mice that received a mixture of mouse brain gangliosides between 6 and 21 d of age and tested in a passive avoidance paradigm performed better than controls at 22 and 32 d of age. However, the performance of treated mice was much worse than controls at 62 and 110 d of age. Therefore, the improvement noted by Karpiak et al. (1984) could have been transient. Jonsson et al. (1984) found that G_{M1} administered daily for the first 4 d of life had no effect on the development of serotonergic neurons in brain up to 2 mo of age. However, such treatment had a preventive effect on degeneration and a stimulatory effect on neuritic growth of serotonergic neurons in animals treated with 5,7-dihydroxytryptamine. It should be emphasized that the treatment period was very short and the long-range effects of ganglioside administration not studied.

Kasarskis et al. (1981) injected 5-d-old rats intracisternally with antiganglioside antibody. When tested at 60 d of age, treated rats had impaired performance in a complex learning task. Their brains had less ganglioside, cerebroside, and RNA, and cortical pyramidal neurons had 31% fewer dendritic spines, the majority of which had a stubby configuration compared with thin spines of control rat brains. The authors proposed that the antiganglioside antibody inhibited neuronal sprouting by binding to the growth cone and so prevented normal dendritic arborization. This is consistent with the findings of Pfenninger and Maylie-Pfenninger (1981a,b) that the glycoconjugate composition of the growth cone is different than elsewhere on the neuron, and that new glycoconjugates are continually being inserted into the growth cone. These combined results strongly suggest that inhibition of brain glycoconjugate synthesis during development could inhibit neuritic sprouting and synaptogenesis and cause neurological impairment. The morphological studies of Purpura (Purpura, 1975; Purpura and Baker, 1977; Purpura and Suzuki, 1976) on brains of humans and animals suffering from ganglioside storage disor-

ders show that endogenously synthesized gangliosides also affect neuritogenesis and synaptic spine formation. Such neurons have large meganeurites with secondary neurites and synaptic complexes that are felt to contribute to the neuronal dysfunction seen in those disorders.

The effect of G_{M1} on induction of cerebral edema has been studied (Karpiak and Mahadik, 1984). Rats received G_{M1} for 2 d before and on the day of mechanical trauma. One day later there was slightly less water in the traumatized region. From this it was suggested that insertion of exogenous gangliosides into neural membranes may alter some of the biochemical mechanisms, resulting in edema. Reduction of edema could play a role in some of the beneficial effects of gangliosides on recovery of neural trauma.

Clinical studies on the effects of ganglioside therapy on several disorders of the human CNS are in progress. Although the published results of most of them are still not available, the possibility of some beneficial effects of ganglioside therapy on stroke (Bassi et al., 1984) indicate that this area of research warrants further investigation.

POSSIBLE BIOCHEMICAL MECHANISMS OF THE GANGLIOSIDE EFFECT

The mechanisms through which gangliosides exert their effects are not known, but undoubtedly involve several biochemical processes. Gangliosides in solution either as monomers or micelles become associated with both biological and artificial membranes (Leon et al., 1981a,b; Schwartzmann et al., 1983; Sharom and Grant, 1978). A portion becomes attached to the surface in micellar form and is easily removed by washing; the remainder becomes more firmly attached. The latter either binds to membrane proteins or becomes anchored in hydrophobic parts of the membrane (Faci et al., 1984; Radsak et al., 1982). Studies using several techniques have shown that gangliosides can affect membrane order (Bertoli et al., 1981; Ishida et al., 1981; Tilack et al., 1982). The ceramide portion alters the packing density of the hydrophobic membrane core and the oligosaccharide chain can affect the order of the cell surface (Sharom and Grant, 1978). The latter effect is probably exerted both through formation of hydrogen bonds among the carbohydrates as well as crosslinking of carboxyl residues through divalent cations. Consistent with this are the findings using freeze-etch electron microscopy that gangliosides tend to form clusters in artificial membranes (Peters et al., 1984). Further evidence that the ganglioside content of biological membranes can affect their order was obtained by studying synaptosomes from cats with G_{M1}- or G_{M2}-gangliosidoses (Wood et al., 1985). Fluorescence polarization studies confirmed that fluidity of synaptosome membranes decreases during normal development. At 2 d of age, polarization profiles of synaptosomal membranes were similar in normal and G_{M1}-

gangliosidosis kittens. However, at 48 d, the profiles of G_{M1}-mutant cats indicated that their synaptosomal membranes were considerably more rigid than those of controls. It is possible that some of the ganglioside effects, such as membrane ruffling, spine formation, and alterations of enzyme activities, could be caused by their effects on membrane order.

The influence of exogenous gangliosides on the activities of several enzymes has been studied. Activity of Na^+,K^+-ATPase in a crude mitochondrial preparation of rat brain was altered when incubated with low nanomolar concentrations of gangliosides (Leon et al., 1981a). Above 50 n*M*, G_{M1} activation of the enzyme was considerably less. In contrast to higher ganglioside concentrations, at the lower concentrations the exogenous ganglioside became firmly bound to the membranes, and it was suggested that such binding altered the enzyme activity by modifying the microenvironment of the membrane around the enzyme. The activity of Na^+,K^+-ATPase in synaptosomes from G_{M1}-mutant cats was not significantly different than controls (Wood et al., 1985). This might be thought to be contradictory to the findings of Leon et al. (1981a) because the concentration of ganglioside within the synaptic plasma membranes of G_{M1} mutants was 24-field higher than normal. However, this lack of a change in activity is consistent with the findings of Leon et al. (1981a) when they incubated their preparations with gangliosides at concentrations greater than 50 n*M* and found progressively less enzyme activation.

The activities of adenylate cyclase (Partington and Daly, 1979) and both calcium-dependent and -independent 3′,5′-cyclic nucleotide phosphodiesterase (Davis and Daly, 1980) activities in rat brain are elevated by exogenous gangliosides. Of considerable related interest is the recent finding that phosphorylation of several membrane proteins in rat brain is modulated by ganglioside–Ca^{2+} complexes (Goldenring et al., 1985). The phosphorylation of some proteins was stimulated, but phosphorylation of others was inhibited. Thus, gangliosides may exert some of their effects indirectly through the cyclic nucleotide systems or directly through the regulation of membrane-bound kinase activities.

Gangliosides have also been shown to affect several neurobiological functions in vitro, including electrical properties of brain slices (Janigro et al., 1984), synthesis, storage, and binding of serotonin (Tamir, 1981; Bach and Sela, 1980) and altered dopamine release from synaptosomes (Cumar et al., 1978). It is possible that gangliosides may be altering similar biological properties during growth and regeneration of nervous tissues.

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