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Review

Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins

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Abstract. Gangliosides, a heterogeneous family of glycosphingolipids abundant in the brain, have been shown to affect neuronal plasticity during development, adulthood and aging. This review will examine old and recent evidence that exogenous gangliosides and in particular GM1, the prototype member of this family, exhibit multimodal neurotrophic effects. Since these compounds are a potential therapeutic tool for the treatment of various forms of acute or chronic neurodegenerative diseases, understanding the dynamic interplay of gangliosides and neuronal cells is essential in the effort to cure neurological disorders. Focus will be given to the novel and provocative hypothesis that gangliosides' neuroprotective properties may derive from their ability to mimic endogenous neurotrophic factors.

Key words. BDNF; GM1; LIGA20; NGF; NT-3; Parkinson's disease; stroke, Trk.

Gangliosides: Biosynthesis, Structure and Nomenclature

The purpose of this article is to provide a brief overview of evidence supporting a role for gangliosides in neuronal plasticity. However, some information about their structure and biosynthesis is required to understand their functional significance.

Gangliosides are classified as acidic glycosphingolipids because they contain sialic acid linked to an oligoglycosyl backbone attached to a ceramide base [1] (fig. 1). They are initially synthesized by the modification of serine to 3-ketosphinganine followed by the addition of various sugar groups, always including at least one sialic acid residue [1]. The sialic acid is usually N-acetyl-neuraminic acid, but it can also be N-glycolyl-neuraminic acid [2]. The synthesis of ceramide appears to take place in the endoplasmic reticulum, while most of the subsequent glycosylations will take place in various compartments of the Golgi apparatus [3]. Following synthesis, gangliosides are transported to the outer leaflet of the plasma membrane, and are found almost exclusively in this region of the cell (very low concentrations of gangliosides have been found in the endoplasmic reticulum, the Golgi apparatus and lysosomes). Their charged sialic acid-containing region projects into the extracellular space, while the nonpolar regions remain inserted in the plasma membrane [4]. Exogenous gangliosides added to culture media may mimic endogenous gangliosides by binding to the cells through insertion into the membrane or by adhering as micelles.

There are many types of gangliosides potentially produced by a cell, and this heterogeneity is due to the various combinations of sugar residues which are subsequently attached to the ceramide base. These sugars can include glucose, galactose, N-acetylgalactosamine, N-acetyglucosamine and fructose [5]. Particular oligosaccharide sequences attached to the ceramide base can be

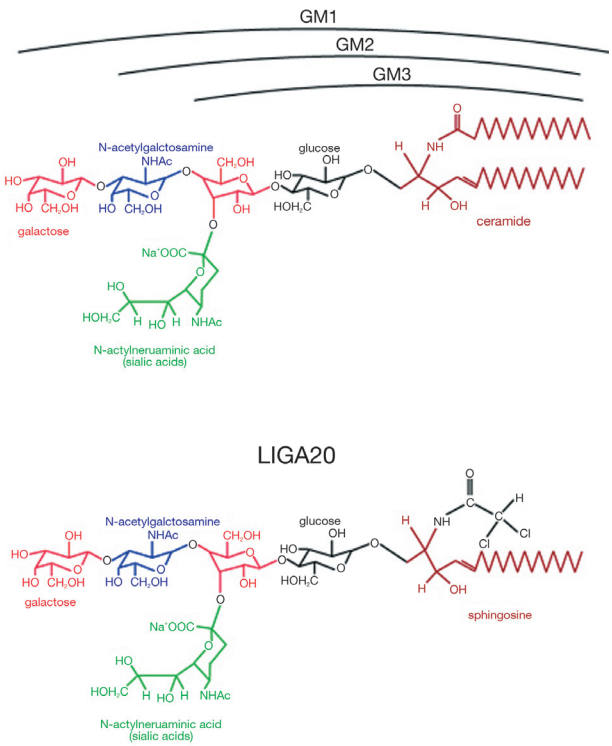

Figure 1. Chemical structure of ganglioside derivatives of acethylneuraminic acid (sialic acid) and ceramide. Four carbohydrates are present in GM1, one glucose, two galactoses and one N-acethylgalactosamine. In GM2 and GM3, the first galactose and the N-acethylgalactosamine are lost, respectively. LIGA 20 is a semisynthetic derivative of GM1 modified in the ceramide tail.

used to identify five categories of gangliosides: ganglio, gala, globo, lacto and hemato. The number of sialic acid residues, which is also used to classify the different ganglioside species, is designated by M (monosialo), D (disialo), and so on [5]. Thus, GM1 (monosialotetrahexosylganglioside), the prototype ganglioside, indicates a member of the ganglio series of gangliosides which contains one sialic acid residue (fig. 1).

Gangliosides and neuronal Plasticity: historical background

Gangliosides were first identified in the 1930s by Ernst Klenk, who suggested the name gangliosides due to the association of these compounds with brain gray matter or *Ganglionzellen* [6]. During the 1950s and 1960s a number of studies confirmed that gangliosides are abundant in the brain in neural cells (neurons and glia) [7, 8], but also in all known vertebrate tissues [9,10]. The omnipresence of gangliosides in all cell types suggests that they are critical in cell physiology. However, their high concentration in developing and adult neurons, up to 10% of a neuron's total lipid content [11], appears to indicate a crucial role for gangliosides in the nervous system.

The pioneering studies of Svennerholm [8] and Suzuki [12] demonstrated a profound variability in the types and amounts of brain gangliosides which appear during mammalian development. The developmental changes in neural gangliosides have been further characterized by a number of investigators [13–19]. Most important, the presence of gangliosides in the nervous system has led most investigations to study and characterize their potential role(s) in modulating brain plasticity and recovery of function.

The therapeutic potential of gangliosides was first explored in studies performed more than 30 years ago. Early investigations by Van Heyningen [20] attributing the effects of tetanus toxin on the central nervous system (CNS) to an interaction with gangliosides led McIlwain [21] to demonstrate that gangliosides can restore the excitability of cerebral tissues left in cold media. In 1976, there were notable studies which stimulated a great deal of interest in the pharmacotherapeutic uses of gangliosides. Purpura and Suzuki [22] performed careful morphological analyses of post-mortem tissues from patients with lysosomal storage diseases. In some of these cases, there was an abnormal accumulation of gangliosides in the brains of the patients due to the loss of activity of specific lysosomal hydrolases, leading to lethal neurological damage. Golgi staining of brain sections indicated the presence of bizarre neuritic outgrowths termed meganeurites. These formations occurred between the cell body and the axon, and were usually many times the area of the cell body. In addition, meganeurites contained what appeared to be dendritic spines, which was quite abnormal considering that these structures were part of the axon. There were also secondary neurites associated with these formations in some cases [22]. These studies appeared to indicate that the abnormal accumulation of glycosphingolipids could profoundly influence the development and/or differentiation of neurons.

Ceccarelli et al. [23] performed the first in vivo test of the ability of exogenous gangliosides to promote the regrowth of damaged neurons. By using a model of preganglionic and postganglionic anastomosis of the feline superior cervical ganglion, these investigators were able to demonstrate that intraperitoneal administration of a ganglioside mixture was able to accelerate the functional recovery of the damaged nerves [23]. In addition, the fact that the preganglionic fibers were cholinergic and the postganglionic fibers were adrenergic suggested that exogenously administered gangliosides were able to affect different classes of neurons [23]. Indeed, a plethora of evidence later confirmed that gangliosides affect multiple neuronal populations. These include dopaminergic, cholinergic, glutamatergic, serotonergic

and noradrenergic neurons (reviewed in [24]). These findings prompted studies to examine the potential use of exogenous gangliosides as therapeutic agents for neurological diseases.

Exogenous gangliosides and neurological diseases

Chronic degeneration

The positive effect of gangliosides in neuronal regeneration and plasticity [22, 23] provoked a great deal of interest in examining their therapeutic use in neurological disorders. Most of the chronic neurodegenerative diseases are characterized by a slow but progressive loss of neurons. Parkinson's disease (PD), for instance, is associated with the loss of dopaminergic neurons in the nigrostriatal system, Alzheimer's disease (AD) with cholinergic neurons of the basal forebrain. Animal models have been generated to recapitulate the motor and cognitive impairments seen in patients with these diseases. These models include mechanical and chemical lesions of specific pathways relevant for PD and AD, the nigrostriatal fibers and fimbria fornix, respectively. Administration of GM1 to rodents exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxidopamine (6-OHDA), two neurotoxins that elicit Parkinson-like signs by inducing the degeneration of dopaminergic neurons in the substantia nigra, stimulated the regeneration of these neurons and ameliorated the abnormal motor responses [25–29]. Importantly, the rescue effect of GM1 on dopaminergic neurons was also observed in nonhuman primates [30, 31]. These preclinical findings led to a small (~50 patients) clinical trial of GM1 in patients with PD. These patients responded to the treatment with an overall improvement of motor function, including decreased rigidity and bradykinesia [32, 33]. Although we are waiting for the conclusion of a larger clinical trial, these data demonstrate the usefulness of GM1 as a treatment for PD.

The prospect that exogenous gangliosides could be exploited for therapeutic purposes has also been assessed in other models of chronic neurodegenerative diseases, such as AD. One of the characteristics of AD is the atrophy of cholinergic neurons of the basal forebrain. The basal forebrain cholinergic system consists of acetylcholine-synthesizing neurons distributed across several distinct areas. These include the medial septal nucleus, the vertical and horizontal limbs of the diagonal band of Broca, the magnocellular preoptic area, the substantia innominata, the nucleus basalis of Meynert and the nucleus of the ansa lenticularis. Experimental evidence has shown that gangliosides facilitate cholinergic reinnervation after lesions of the septal nuclei or of the nucleus basalis, or after transection of the fimbria-fornix [34–38]. In addition, GM1 exhibited neuroprotection and promoted reinnervation of cholinergic neurons after cortical infarction, which produces well-defined anatomical and biochemical deficits of the nucleus basalis in rats as well as nonhuman primates [39–42]. Remarkably, GM1 can also promote morphological and functional recovery in normal aged animals. In fact, both morphological and behavioral data have shown that the degeneration of cholinergic neurons typically associated with aging is attenuated by GM1 [43–45]. Overall, the studies in animal models of chronic neurodegenerative diseases support a role for GM1 as a neuroprotective and neurotrophic agent.

Apoptosis, ischemic stroke and spinal cord injury

A common feature seen in chronic neurodegenerative diseases is apoptosis, a form of programmed cell death believed to be caused by secondary injury processes [46]. Apoptotic neurons, however, can be rescued as they remain viable for some time. Thus, there is a need for antiapoptotic agents to rescue injured neurons. Are exogenous gangliosides neuroprotective because they reduce apoptosis? The answer appears to be yes. In fact, studies from different laboratories have established that gangliosides possess antiapoptotic effects in a variety of experimental models, such as growth factor deprivation [47], low potassium [48], ethanol [49] and glutamate [50, 51]. These data raise a salient point that gangliosides may be useful in a wide range of neuronal diseases characterized by apoptosis, including stroke.

Current experimental strategies to limit the incidence of stroke in the CNS are focused on the first or recurrent strokes by reducing risk factors (e.g. high blood pressure). While such an approach may be beneficial, it is clear that other strategies should be developed, such as minimizing neuronal damage. It has been suggested that glutamate, released during brain ischemia/hypoxia, can induce secondary neuronal injury via an apoptotic pathway. Similarly, injury to the spinal cord triggers an abnormal release of glutamate and other excitatory amino acids that contribute significantly to the neurological outcome. Thus, anti-excitotoxic or anti-apoptotic agents are viewed as potential therapies against the neuropathological consequences of stroke and trauma. Anti-excitotoxic compounds, however, exhibit side effects which limit their clinical use. Exogenous gangliosides appear to be a valid alternative therapy as anti-excitotoxic compounds. In fact, GM1, GD1b and GT1b have been shown to effectively block glutamate excitotoxicity in vitro, [52–54] and to limit the severity of ischemic brain lesions after experimental stroke [55–57]. The neuroprotective effect does not appear to result from direct inhibition of glutamate receptor function, as indicated by an absence of changes in glutamate-mediated ionic conductance following incubation with gangliosides [51, 52, 58]. Rather, it appears that gangliosides act on both intracellular and

extracellular events involved in apoptosis, which will be presented later.

The use of gangliosides in humans is still limited despite the positive outcome of clinical trials showing that GM1 and other gangliosides, either alone or in combination with other neuroprotective agents, reduce neuronal damage after stroke [59] and spinal cord trauma [60]. Major setbacks preventing a wide use of gangliosides in human neurodegenerative disorders are concerns about side effects, such as the potential of GM1 to cause allergic reactions [61] or an acute Guillain-Barré syndrome [62, 63]. However, these cases were sporadic and uncommon in large clinical trials [64] and also occurred after ganglioside withdrawal [65]. In addition, because GM1 used for clinical studies has been extracted and purified from bovine brain, there is still apprehension that it can be contaminated by prions (or other viruses) that cause bovine spongiform encephalopathy. This can be easily avoided by extracting gangliosides from pig brain or designing new procedures to extract viral-free GM1 from cattle brain [66]. Nevertheless, evidence that GM1 is safe is overwhelming. Thus, clinicians should be encouraged to consider gangliosides as potential therapy for acute stroke or trauma.

Semisynthetic gangliosides

GM1 is efficacious in animal models of neurodegenerative diseases. Combined with evidence that lower levels of endogenous gangliosides inhibit nerve regeneration and induce axonal damage [67, 68], and that individuals that do not synthesize GM3 gangliosides suffer seizures [69], this evidence should encourage more clinical investigators to consider applying ganglioside to a variety of neurological disorders. As no therapies are still currently available to reduce atrophy and loss of CNS neurons, gangliosides would be a welcome therapy. However, gangliosides administered by mouth are rapidly inactivated. When given systemically, they insert and concentrate in neuronal membranes very slowly. Thus, gangliosides may have a limited therapeutic application for human diseases. These considerations prompted the synthesis of ganglioside derivates having properties similar to natural gangliosides but possessing physicochemical characteristics for oral administration and facilitated rates of insertion into neuronal membranes.

Relatively few classes of semisynthetic gangliosides have been characterized. These include derivatives of sphingosine or LIGA [52]. The LIGA analogs have the basic structure of GM1, but the ceramide portion is modified so that the fatty acid tail at the 2-amino position is substituted by acetyl (LIGA4) or dichloroacetyl (LIGA20) groups (fig. 1). These compounds possess very rapid onset of action. In the case of LIGA20,it is more potent than GM1 [52, 70, 71], can be administered by mouth [71–73] and has fewer side effects [74, 75]. Moreover, because of the preparation and purification procedures, these compounds may be less likely to be contaminated by proteins or infectious agents from the cattle brains. Therefore, semisynthetic ganglioside may be a more suitable alternative for preventing or slow down neurodegenerative processes in humans.

Mechanisms of action

The plethora of effects of gangliosides in the nervous system has been confounded by the inability to define a clear mechanism of action. This is mostly due to the fact that exogenous and endogenous gangliosides do not share the same effects. Thus, it is important to distinguish the physiology of gangliosides from their pharmacology. The subcellular localization of gangliosides would suggest an interaction between gangliosides and the extracellular milieu, and the ongoing characterization of these compounds has mostly supported this notion. For example, GM1 can serve as a receptor for cholera toxin [76] and appears to be absolutely necessary for internalization of this toxin [77, 78], while ganglioside GT1b appears to be involved in the effects of tetanus toxin on the brain [79]. The known extracellular roles of gangliosides are complemented by studies indicating that they may serve as reservoirs of bioactive products which can profoundly affect cell function. These breakdown products include ceramide, sphingosine, sphingosine-1-phosphate, as well as other ganglioside derivatives [2, 80–82]. On the other hand, ceramide is often considered a downstream mediator of various apoptotic signal transduction cascades [83, 84]. There is also evidence in hematopoietic cells that GD3 ganglioside can be a downstream agent of ceramide-induced apoptosis [85–87]. Thus, ceramide may not modulate the neuroprotective properties of gangliosides.

Endogenous gangliosides may be involved in the formation of glycosphingolipid rafts in cell membranes. It has been suggested that these domains serve as platforms for bringing various signaling proteins together (i. e., receptors and their targets) [88, 89]. These rafts may also serve to sort proteins in the trans-Golgi network to particular domains of the cell [89]. Additionally, Hakomori and Handa [90] have suggested a function of detergent-insoluble glycosphingolipid-enriched microdomain (DIGEM) as direct signaling receptors, which, upon interaction with various lectins, can transduce a signal intracellularly through DIGEM-associated signaling factors. The ganglioside-specific sialidase Neu3, which has been shown to act as a transducer molecule [91], may also participate in the neurotrophic mechanisms of gangliosides. Thus, gangliosides may be able to modulate cell function through a variety of avenues. Nevertheless, none of the above theories may fully explain the ability of exogenous gangliosides to improve neuronal plasticity.

Table 1. Neuroprotective effects of GM1 and neurotrophins on major neurological disorders and/or experimental models of neurodegenerative diseases.

Diseases/animal models	GM1	BDNF	NGF	NT-3
AIDS dementia*	+	++	–	–
Alzheimer's disease/degeneration of basal forebrain	+	+/–	++	+/–
Motor neuron degeneration/axotomy	+	+	–	+
Parkinson's disease/degeneration of substantia nigra	+	++	–	+
Seizure	+	+	+	–
Spinal cord injury/descending motor pathways	+	+	–	+
Stroke	+	++	+	+/–

+ or – denotes the neuroprotective effect: ++ robust, + mild, +/– weak, - no effect. *Unpublished. For appropriate references see text.

There are a number of viable theories to account for the pharmacological use of exogenous gangliosides, including (i) Effects on intracellular calcium, (ii) activation of intracellular enzymes and (iii) modulation of receptors. These theories will not be presented because they have already been reviewed elsewhere [92–96]. Instead, to provide a valid alternative explanation of the neurotrophic theory of gangliosides, I will discuss the interaction of gangliosides with the neurotrophins (NTs).

Ganglioside properties are similar to those of NTs

The NT family of neurotrophic factors includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. These small, basic, homodimeric proteins are required for the growth, differentiation and survival of specific but overlapping neuronal populations. Because of space constraints, comparatively little of the biology of NTs and their receptors themselves are critically examined in this review, which focuses instead on the effects of NTs that may be relevant to gangliosides. Interested readers are referred to many excellent reviews on the biological actions of NTs [97–100].

One of the crucial aspects of the neurobiology of the NTs is their ability to prevent damage to CNS neurons caused by neurotoxic agents, trauma and ischemia. For instance, NGF reduces the atrophy of cholinergic neurons and the associated impairment in learning and memory occurring either following a lesion of the cholinergic septohippocampal projection or in age-impaired rats [101]. BDNF has similar effects, although with a lower potency [102]. On the other hand, BDNF protects the nigro-striatal pathway against the toxic property of MPTP or 6-OHDA [103–105]. Similarly, NGF prevents 6-OHDA mediated oxidative stress in PC12 cells [106]. Both BDNF and NT-3 can promote sprouting of corticospinal neurons after spinal cord injury [107], and prevent the retrograde cell death of axotomized red nucleus in developing rats [108]. BDNF has also been shown to reduce secondary injury caused by ischemia [109]. The neuroprotective activity of the NTs has also been extended to neurotoxins in vitro. These include glutamate [110, 111], amyloid [112], ethanol [113] and human immunodeficiency virus glyprotein 120 [114]. Significantly, most of the trophic effects of NTs mentioned above can be reproduced using GM1 or other gangliosides, although the NTs are more potent pharmacologically (table 1).

Gangliosides and the Trk receptors

The trophic and neuroprotective properties of gangliosides such as GM1 appear to demonstrate striking (although not identical) similarities to the NTs, and therefore suggest a similar mechanism of action. NTs bind to two types of receptors: transmembrane tyrosine kinase receptors (Trks) and the p75 neurotrophin receptor (p75NTR) [115]. The three most well characterized Trk family members include TrkA, TrkB, TrkC, where NGF binds primarily to TrkA, BDNF and NT-4/5 bind to TrkB, while NT-3 binds primarily to TrkC, but can also activate TrkA and TrkB [98]. The p75NTR binds all NTs with relatively equal affinity. Trks are 140-kDa proteins possessing intrinsic tyrosine kinase activity which can be stimulated by ligand binding, resulting in dimerization of the receptors [116]. The subsequent phosphorylation of intracellular target proteins such as phospholipase c-γ (PLCγ), extracellular signal-regulated kinase (Erk1/2), phosphatidylinositol-3-kinase/Akt and Suc-associated neurotrophic factor target (SNT) initiates a cascade of events resulting in growth, differentiation or survival [117]. Most of the trophic effects of NTs are mediated by Trks, although there is evidence indicating that the p75NTR can potentiate Trk's activity, affect the specificity of Trk binding and mediate some responses, such as apoptosis, in the absence of Trk [118].

Different laboratories have independently shown that GM1 activates TrkA via tyrosine phosphorylation of its tyrosine kinase domain [119–121]. This event is followed by TrkA dimerization [122] and activation of a number of

target proteins, including PLCγ, Erk1/2 and SNT [121, 123]. The ability of GM1 to induce TrkA tyrosine phosphorylation helps elucidate the variety of intracellular events attributed to GM1 and perhaps other gangliosides. For instance, TrkA activation by GM1 may explain the positive effect of this ganglioside on the induction of cholinergic parameters such as choline acetyltransferase activity and choline uptake, and it is reminiscent of the effect of NGF [38, 124]. Activation of TrkA, known to promote accumulation of intracellular Ca^{2+} [125], may explain ganglioside induction of the Ca^{2+}-dependent protein kinase [126] and changes in intracellular Ca^{2+} concentrations [58, 127]. SNT phosphorylation, which is associated with neurite outgrowth and somatic hypertrophy in PC12 cells, may help clarify the ability of GM1 to potentiate the neuritogenic effects of NGF as well as to act as a survival factor [128] in these cells. Moreover, activation of Erk phosphorylation by GM1 is likely to underlie its neuroprotective property in stroke, as BDNF-mediated induction of the Erk pathway has been shown to reduce ischemic neuronal damage [129]. However, since the metabolism of gangliosides may result in the production of metabolically active byproducts such as sphingosine and ceramide, it is believed that the entire range of properties exhibited by gangliosides is probably not explainable by activation of TrkA alone. Moreover, GM1 or other gangliosides exert neurotrophic activity on neurons that do not respond to NGF activation of TrkA [24]. For instance, the neuroprotective activity of LIGA20 on cerebellar granule cells is mediated by TrkB and not TrkA activation [75]. These considerations prompted exploration of the interaction of gangliosides with each of the Trk neurotrophin receptors.

Studies aimed at determining the relative potency of GM1 on Trk tyrosine phosphorylation have proven that gangliosides activate different Trks. In fact, GM1 is more potent in activating TrkC tyrosine phosphorylation than TrkA or TrkB [130]. Due to the abundant distribution of Trks and neurotrophins in the PNS and CNS, these findings shed light on the broad and overlapping trophic activity of exogenous gangliosides. Such complexity might be critical for generating the remarkable diversity and specificity with which gangliosides regulate neuronal function.

Gangliosides induce the release of NTs

The interaction of GM1 with more than one Trk receptor may explain the discrepancies between the range of biological effects modulated by gangliosides and the restricted distribution of TrkA-responsive neurons in the nervous system. For instance, the trophic effect of GM1 on PC12 cells is instructive in demonstrating the contrasting biological activity of gangliosides in different cellular environments. In these cells, GM1 ganglioside is able to promote survival but not differentiation [131], a property of NT-3. However, GM1 can induce the differentiation of other cells, i. e. neuroblastoma cells [132, 133], which are responsive to BDNF. How can glycosphingolipids simultaneously activate different Trks given the fact that each neurotrophin has a different affinity for these receptors? The answer appears to lie in the ability of gangliosides to induce the release of NTs. GM1, in fact, has been shown to increase the release of NT-3 and NGF in non-neuronal cells [130, 134], while LIGA20 induces the release of BDNF from cerebellar granule cells [75, 130]. To understand the importance of these findings in relation to synaptic plasticity, a brief overview of the NT release field is necessary.

NTs are stored in large dense-core vesicles from which they can be released by constitutive or activity-dependent secretory pathways [135, 136]. Neurons of both, the peripheral nervous system (PNS) [137] and the CNS [111, 138], as well as non-neuronal cells, release NTs. Released NTs are able to activate Trks on the neurons themselves, through an autocrine mechanism [111, 137, 139], or activate a cognate receptor through a paracrine mechanism. As mentioned above, while BDNF binds more selectively to TrkB, NT-3, in the absence of p75NTR, activates TrkA and TrkB in addition to TrkC [140]. This lack of selectivity for a particular Trk receptor is pharmacologically important because it increases the possible combination of ligand-receptor interactions and consequently the number of neurons that can be affected by the NTs. In addition, NTs are released from both synapses and extrasynaptic sites. While the latter affects dendritic branching [141], NTs released from synapses modulate both presynaptic and postsynaptic events, including neurotransmitter release and synthesis, and receptor function [136, 142]. Reduced release of NTs causes severe deficits in synaptic potentiation and stabilization, which have been shown to contribute to the pathogenesis of neurodegenerative diseases [143, 144]. Therefore, compounds such as gangliosides, which induce the release of NTs, are crucial for the proper dynamic of neuronal plasticity.

The ability of exogenous gangliosides to exert trophic activity in a variety of neuronal cells could be explained by their relative potency in affecting the constitutive and activity-dependent secretory pathways of NTs. For instance, the effect of GM1 on NT-3 secretion may explain why GM1 can promote the survival of PC12 cells but is unable to promote neurite outgrowth from these cells [119]. The NT-3 released from these cells [145] would only be able to weakly activate the TrkA receptor, as has been demonstrated, resulting in survival but not differentiation of the treated cells. The ability of GM1 to induce the differentiation of other cell lines, such as neuroblastomas [146], can potentially be due to fact that, unlike PC12 cells, neuroblastomas produce NTs [147].

Thus, the released NT-3 could synergize with the NTs already secreted by these cells, resulting in differentiation. Similarly, the weak activity of GM1 as compared with LIGA20 in preventing glutamate toxicity [52] could be explained by the relative potency of GM1 and LIGA20 to release BDNF [75], which among the NTs is the strongest anti-glutamatergic factor.

Potential mechanisms

The autocrine mechanism of NT regulation provides a link between the ability of gangliosides to induce the release of NTs and to activate Trk tyrosine phosphorylation. However, the molecular mechanism(s) whereby gangliosides induce the release of NTs is still a matter of speculation. Presynaptic release of NTs is modulated by increased intracellular Ca^{2+} either from opening Ca^{2+} channels or mobilization from internal stores [148, 149]. As mentioned before, GM1 has been shown to increase intracellular Ca^{2+} levels in neurons via Ca^{2+} channels [150–152], making the Ca^{2+} influx hypothesis an attractive possibility to explain the ability of gangliosides to release NTs (fig. 2). Thus, it may be possible that gangliosides may induce the release of NTs from neurons or glial cells in a Ca^{2+}-dependent manner. On the other hand, GM1 induces the release of NTs in non-neuronal cells as well [130] suggesting a Ca^{2+}-independent mechanism.

Gangliosides, like other glycosphingolipids, are able to form protein-lipid interactions and as, mentioned before, participate in the formation of membrane microdomains such as glycosphingolipid-based membrane rafts or bind to VIP21-caveolin within the plasma membrane [153]. Caveolin is a membrane protein distinct from the coated pit and localized in caveolae [154], a plasma membrane invagination rich in cholesterol and sphingolipids and specialized in the cellular transport of molecules. Caveolin has been shown to exert an important function as an anchoring protein for signaling molecules, including various kinases, tyrosine kinase receptors, Trk and p75NTR within the plasma membrane [155–157]. By interacting with caveolin, gangliosides may enhance or inhibit the activity of these molecules, which, in turn, may promote the release of NT-3. Alternatively, gangliosides, though their lipid moiety, may interact with synaptophysin or other proteins that are essential for synaptic vesicles [158], and modulate the fusion of vesicles with membranes and the release of their content. Therefore, it is possible that multiple mechanisms control ganglioside-mediated release of NTs.

Gangliosides modulate NT synthesis

In addition to modulating their own secretion [139, 159], NTs are also capable of upregulating their own synthesis in a Trk-mediated manner [134, 145, 149]. This event is essential to avoid depleting intracellular stores and keep a ready supply of NTs. Studies aimed at testing whether gangliosides regulate NT expression as well as NT secretion revealed that GM1 increases NGF messenger RNA (mRNA) in rats [160] as well as in vitro [134]. The effect is mediated by Trk because Trk-positive cells exposed to GM1 exhibited higher levels of NGF mRNA and protein than Trk-negative cells or cells exposed to control medium [134]. Therefore GM1, by modulating constitutive or activity-dependent release of NTs, activates Trk receptors by an autocrine loop, which in turn triggers a positive feedback to increase their synthesis. It is reasonable to speculate that gangliosides positively modulate the interplay between NT release and synthesis, a major departure from the classical view that signaling molecules released from neurons provide a negative feedback to reduce their synthesis. The reciprocal induction of NT synthesis by secreted NTs suggests a novel molecular framework by which gangliosides might influence synaptic plasticity. It remains to be established whether the GM1-mediated autocrine/paracrine interaction between NTs and their re-

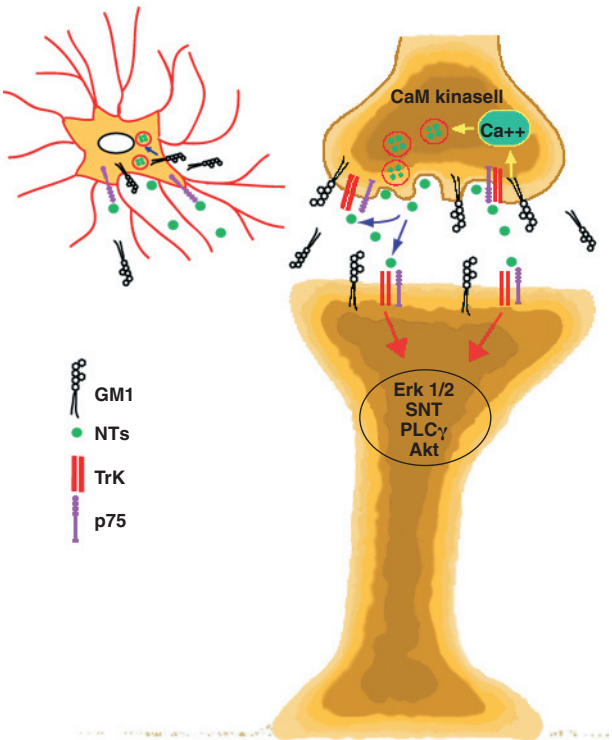

Figure 2. Suggested mechanism of NT release by gangliosides. Exogenous GM1 induces Ca^{2+} influx and release of Ca^{2+} from internal stores both in neurons and glial cells. Increased intracellular Ca^{2+} concentrations evoke the release of NTs from vesicular stores. GM1-mediated intracellular Ca^{2+} increase can also activate Ca^{2+}calmodulin-dependent protein kinase II, which may induce directly the release of NTs from vesicles or through Ca^{2+} mobilization from internal stores. Once released, NTs activate presynaptic NT receptors or diffuse across the synaptic cleft and activate postsynaptic NT receptors and related signaling proteins (e. g. ERK1/2, Akt).

ceptors occurs in multiple cell populations. Nevertheless, the overlapping distribution of NTs with their receptors supports the broad action of gangliosides.

Conclusions

Exogenous gangliosides have been used as an experimental tool to promote the recovery of function of various types of neurons following neuronal damage. Although there is evidence that GM1 ganglioside can promote the survival and/or regrowth of injured neurons, the specific mechanism of action of gangliosides has been difficult to define. It is now recognized that gangliosides can potently, although indirectly, activate Trk receptors by enhancing the endogenous release of NTs, resulting in autocrine and paracrine activation of Trk receptors and associated signaling molecules (fig. 2).

The ability of gangliosides to induce the release of NTs from different cell types appears to explain the spectrum of effects seen by gangliosides on different neuronal populations. The fact that NT-3 can interact with different Trk family members, as well as potentiate the effects of NGF and possibly BDNF, would allow GM1 to be effective on a variety of neuronal populations expressing different NT receptors. Whether GM1 can induce the release of other factors besides NTs requires further examination. In addition, it remains to be established whether gangliosides induce the release of mature fully processed NTs as well as pro-NTs. The pro-NTs preferentially bind to p75NTR and mediate apoptosis [161, 162], which may explain why higher concentrations of gangliosides cause neuronal damage [163] or abolish the trophic effect of NGF [164]. Similarly, it remains to be established whether changes in the types of gangliosides present in the nervous system during neuronal development may modulate levels of NTs being secreted by these cells. Furthermore, the identification of gangliosides in a variety of cell types, both neuronal and non-neuronal, may also be due to a particular role of glycosphingolipids in differential exocytosis. These and other possibilities, which can be extrapolated from experimental evidence, may allow for a greater understanding of the role of these compounds in vivo, as well as their more informed use as neuro-therapeutic drugs.

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