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EVIDENCE FOR NERVE GROWTH FACTOR-GANGLIOSIDE INTERACTION IN FOREBRAIN CHOLINERGIC NEURONS

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Abstract. Cholinergic neurons of the forebrain respond trophically to nerve growth factor (NGF) in some experimental circumstances. The cholinergic cell system of the nucleus basalis magnocellularis (NBM) which projects to the cortex shows signs of cellular degeneration following limited devascularizing cortical lesions, while no apparent damage is observed in the remaining ipsilateral cortex. These cholinergic cells possess receptors for NGF and the administration of this peptide into the cerebroventricular space prevents cell shrinkage and loss of activity of the biosynthetic enzyme for acetylcholine, choline acetyltransferase (ChAT). Analogous trophic responses can be elicited in this system with the application of the sialoganglioside GM_1. In addition, GM_1 can increase the effects of NGF on ChAT activity in lesioned neurons of the NBM-to-cortex model system described above. This cooperative interaction is observed even when ineffective doses of GM_1 are administered. Furthermore, an interaction between these two putative neurotrophic substances has been noted over other cholinergic parameters such as cortical high affinity choline uptake (HACU). These studies confirm the idea that trophic factors can be utilized to rescue degenerating neurons of the CNS and, in addition, lend support to the concept that gangliosides can facilitate actions of endogenously produced trophic factors.

INTRODUCTION

Recent evidence gives credence to the idea that putative trophic substances can be utilized, at least experimentally, to rescue degenerating neurons of the central nervous system. The regenerative potential of the CNS is most likely governed by a cohort of endogenously produced trophic factors (for review see 16). Nerve growth factor (NGF) can be considered as a prototype for this category of neurally produced trophic substances. NGF exerts well defined trophic effects on distinct cells of the nervous system *in vivo* and *in vitro* (22). In addition to NGF's peripheral actions, it acts on subsets of centrally located neurons (19, 23). A number of central forebrain cholinergic neurons in the adult have been shown to be responsive to NGF in some experimental conditions. Indeed, forebrain cholinergic neurons, particularly those of the medial septum and the NBM, contain NGF binding sites (for review see 6, 36, (38). Their terminal target sites have been shown to produce the trophic factor which can be retrogradely transported to cell bodies of these neurons (for review see 36, 38). More importantly, these cholinergic neurons respond trophically to NGF following partial or total damage of the septo-hippocampal connections (13, 14, 19, 39). Sialogangliosides, particularly GM_1, have been reported to exert neurotrophic effects in a variety of *in vivo* and *in vitro* conditions (for review see 3, 21, 30). These glycosphingolipids when applied *in vivo* promote the anterograde regeneration of acetyl-cholinesterase reative fibres in the hippocampus after partial fimbria transections (28) (see also Oderfeld-Nowak and co-workers, this volume). The administration of GM_1 has also been shown to prevent the retrograde cell shrinkage of NBM cholinergic neurons which occurs following limited cortical devascularization (7). The nature of these ganglioside effects on cholinergic neurons and their possible interaction with endogenous trophic substances remains to be established. This short review deals with the actions of NGF and gangliosides on neurons of the NBM-to-cortex cholinergic pathway.

NGF AND GANGLIOSIDE CAN RESCUE DAMAGED CHOLINERGIC NEURONS

Devascularizing lesions of the neocortex result in retrograde cell shrinkage and loss of neurites of the rat NBM cholinergic neurons. These changes become apparent in young and mature rats 30 days post lesion (31). The immunohistochemical signs of neuronal degeneration are accompanied by a pronounced decrease in the ChAT enzymatic activity in microdissected samples of the NBM (34) while no changes are observed in other CNS nuclei examined at various time intervals. We have shown

that these biochemical and anatomical degenerative changes can be completely prevented in cortically lesioned rats by administering large doses (30 mg/kg/day, intraperitoneally (i.p.)) of the sialoganglioside GM_1 for 30 days, beginning immediately post lesion (7). NGF, at very small doses, ensures full protection of NBM cholinergic neurons following injury. We have observed that the intracerebroventricular (i.c.v.) administration of NGF for only 7 days, beginning at the time of lesioning in doses of 12 μg/day prevents the decrease in ChAT activity in the NBM after partial cortical infarction (4, 5). The magnitude of this protective effect was shown to be comparable to that obtained with i.c.v. administration of GM_1 alone (5 mg/kg/day) (Table I). Interestingly, the combined administration of NGF and GM_1 resulted in increased ChAT activity in the NBM ipsilateral to the lesion site, above control levels (Table I). The immunocytochemical analysis also revealed protection of the normal morphological features of these cholinergic neurons and suggests an apparent increase in the number of ChAT immunoreactive processes in the neuropile (Fig. 1).

TABLE I

Effect of NGF administered in combination with an *effective* dose of GM_1 (5 mg/kg/day, 7 days) on ChAT activity in the NBM and cortex of mature rats, 30 days following unilateral decortication

		Ipsilateral NBM		Ipsilateral cortex	
Group	*n*	ChAT activity	% Control	ChAT activity	% Control
Control	6	57.67±3.86	—	35.81 ±2.39	—
Lesion + Vehicle	6	31.16±3.17	54*	35.85 ±1.74	100
Lesion + GM_1	5	61.94±6.55	107	50.70 ±2.44	142*
Lesion + NGF	5	50.94±3.75	88	47.63 ±3.12	132*
Lesion + GM_1 + NGF	5	69.41±1.06	120*	84.82±10.42	237*

Values for ChAT activity are the mean ± SEM, and expressed as nM/mg protein/h; *n* indicates number of cases; *significantly different from control at $p < 0.01$, ANOVA followed by a post-hoc Dunnett's test (4).

ChAT activity in the remaining ipsilateral cortex of lesioned vehicle treated animals did not differ from that of the unlesioned contralateral side. The administration of either NGF or GM_1 resulted in increased ChAT activity in the remaining cortex adjacent to the lesion site over that of control levels. In rats receiving these substances in combination, ChAT activity in the remaining ipsilateral neocortex increased to over 200% of control values, suggesting a cooperativity between these two agents (Table I). The possibility that these two putative neurotrophic

agents might act in a cooperative fashion has been reinforced by studies where cortically lesioned animals were treated with NGF and low, ineffective doses of GM_1. Thus, the application of low doses of GM_1 (0.5 mg/kg/day, i.c.v., 7 days) administered concurrently with effective doses of NGF to cortically lesioned rats resulted in an increase in ChAT activity both in the affected cell body area (NBM) and the terminal network of the ipsilateral neocortex (see Table II).

TABLE II

Effect of NGF administered in combination with an *ineffective* dose of GM_1 (0.5 mg/kg/day, 7 days) on ChAT activity in the NBM and cortex of mature rats, 30 days following unilateral decortication

		Ipsilateral NBM		Ipsilateral cortex	
Group	*n*	ChAT activity	% Control	ChAT activity	% Control
Control	6	69.06±4.67	—	39.20±3.77	—
Lesion + Vehicle	6	44.87±6.60	65*	38.20±4.69	97
Lesion + GM_1	5	46.92±2.80	68*	36.93±2.80	94
Lesion + NGF	5	73.07±3.30	109	59.06±2.90	151*
Lesion + GM_1 + NGF	5	83.87±6.56	121*	72.98±4.08	186*

Values for ChAT activity are the mean ± SEM, and expressed as nM/mg protein/h; *n* indicates number of cases; * significantly different from control at $p < 0.01$, ANOVA followed by a post-hoc Dunnett's test (4).

To examine further the interaction between NGF and GM_1, in particular in the the remaining ipsilateral cortex, we studied their effect on HACU, a marker for cholinergic nerve terminals. The *in vitro* effect of these factors on the uptake process was examined by preincubating cortical synaptosomes from control or lesioned rats with various concentrations of either GM_1 and/or NGF prior to the addition of [^{3}H]choline. These two factors *in vitro* did not directly alter HACU (10). However, following their administration *in vivo* to decorticated rats, for a maximum of 7 days, a time-dependent effect on cortical HACU was observed. Briefly, while cortical HACU did not differ from control values in any of the groups 1 or 5 days post lesion, animals treated with GM_1 or NGF tended to show a rise in synaptosomal HACU by the 15th day post lesion. This increase became more marked and was significantly different from control values at 30 days post lesion (Table III). At these time points, a potentiation by GM_1 of NGF effects on HACU was also observed. Treatment with both GM_1 and NGF significantly augmented cortical synaptosomal HACU by 58% and 85% over control values on

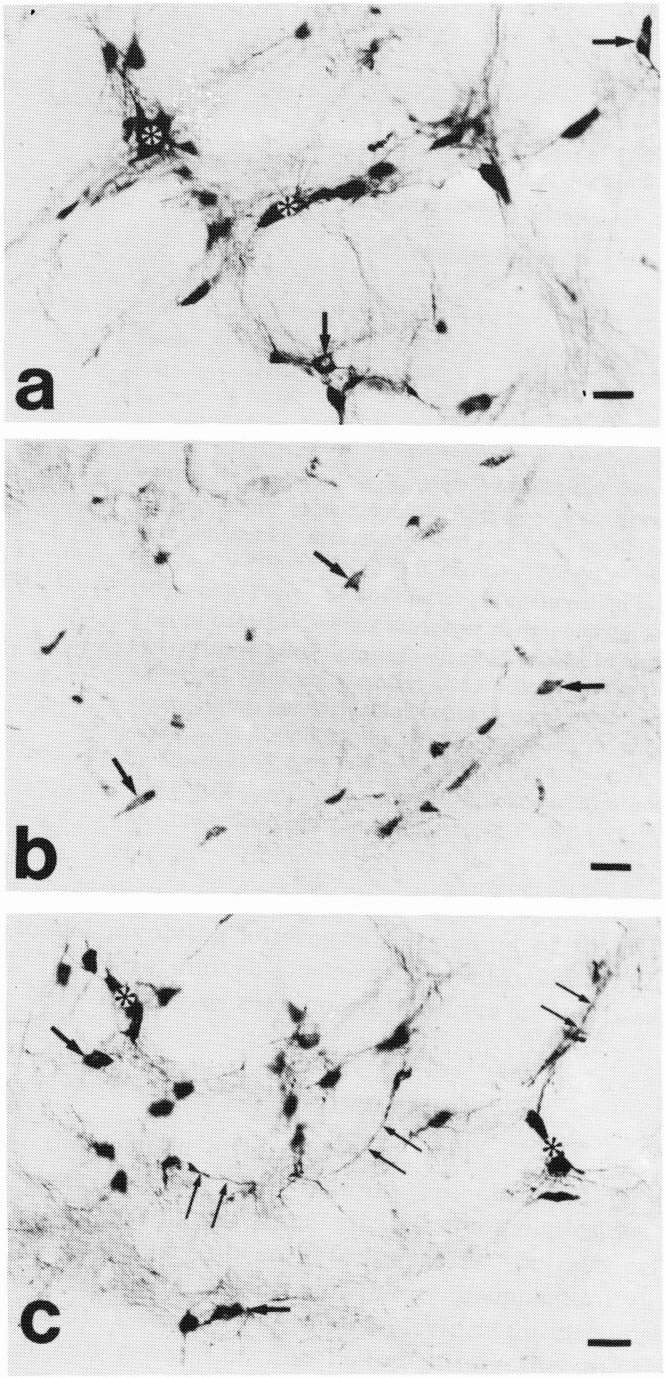

Fig. 1. Appearance of ChAT-immunoreactive neurons in NBM in control (a), lesioned (b), and lesioned GM_1/NGF-treated (c) rats. Clustered (asterisks) and isolated (arrows) cholinergic cell bodies are indicated. Thinner, paired arrows in (c) indicate immunoreactive processes. Note that cell shrinkage is prevented in factor-treated rats. (Interference contrast optics; bar, 25 μm, 4).

TABLE III

Effect of NGF and/or GM_1 on cortical high affinity [^{3}H]choline uptake

Group	Post-lesion day		
	5	15	30
Lesion + vehicle	109	97	98
Lesion + GM_1	102	124	134*
Lesion + NGF	109	121	136*
Lesion + GM_1/NGF	116	158*	185*

Values are expressed as % control (8.80±0.95 pmoles [^{3}H]choline/mg protein/4 min). Rats (n = 6 per group) were unilaterally decorticated and received immediately post-lesion either: GM_1 (1.5 mg/day, 7 days), NGF (12 μg/day, 7 days) or both factors in combination i.c.v. The ipsilateral remaining cortex or corresponding control cortex was homogenized in 0.32 M sucrose in Hepes buffer pH 7.4 (from Garofalo and Cuello, 10). Cortical synaptosomes and high affinity choline uptake were measured using a modification of the procedures described by Simon et al. (29).

* $p < 0.05$ from control ANOVA, followed by a post-hoc Newman-Keuls test.

the 15th and 30th post lesion days, respectively. Using a complementary animal model involving anterograde lesioning of the NBM with ibotenic acid (8), Di Patre and co-workers have also shown that GM_1 can potentiate NGF effects on cholinergic markers in the basalis-cortical pathway.

CONCLUSIONS

While the trophic effects of bona fide neurotrophic substances such as NGF over lesioned CNS neurons are observed in a wide range of experimental paradigms, those elicited by gangliosides require specific conditions. These have been referred to as "permissive" conditions, for the *in vivo* effects (4, 35) or a "window of opportunity" for the *in vitro* effects (37). It is conceivable that under certain circumstances, the availability of endogenous trophic factors alters the ability of cells to respond to gangliosides. The injured brain produces low amounts of endogenous trophic factors immediately after the insult (27). It is, therefore, possible that following extensive neural lesions, CNS neurons are in a rather vulnerable state. Accordingly, reversible or irreversible neuronal degeneration occurs. In central cholinergic neurons, retrograde de-

generative changes can be partially reversed with the timely administration of NGF (15, 20, 39) and, also, some recovery is observed even when initiation of treatment is delayed 21 days post lesion (13). In contrast, in the *in vivo* NBM cholinergic model, early initiation of ganglioside treatment is essential for protection of the neurons (35). After a delay of ten days gangliosides are ineffective in preventing retrograde cholinergic degeneration (35). An analogous observation has been made for the anterograde degeneration of nigral dopaminergic neurons in the MPTP model (11). Therefore, it can be proposed that the *in vivo* administration of gangliosides could prevent neuronal degeneration in the CNS cholinergic system by potentiating the actions of the low amounts of available endogenous trophic factors produced in the first few days after a lesion. The lack of protection offered by exogenous gangliosides on cholinergic neurons in lesioned aged rats (35) can be explained in the same manner since aging is accompanied by an apparent loss of NGF receptors (17) and a diminished production of endogenous factors after injury (26). Another component of the "permissive conditions" which should also be considered is the duration of ganglioside administration. In the cholinergic system, 7 days of i.c.v. administration of GM_1 is adequate for protection against retrograde damage, the effect persisting at least 23 days beyond the cessation of treatment (35). However, a more prolonged treatment seems to be required for protection of cortical noradrenergic fibers affected by 6-OH-dopamine (18) and of dopaminergic neurons in the nigrostriatal system after MPTP lesioning (11, 12).

It is well documented that the actions of NGF are receptor-mediated (for reviews see 36, 38). The actions of gangliosides are largely dependent on the sugar moiety of the sugar-ligand complex (see Ledeen and collaborators, this volume). Many hypotheses have been advanced to explain the mechanism(s) of action through which gangliosides exert their protective or reparative effects in CNS neurons, although they remain undefined. One of the difficulties is that glycosphingolipids cannot be considered in the orthodox context of drug-receptor interactions. However, using classical ligand binding techniques utilizing radiolabelled protein-sugar complexes ("neoganglio proteins"), the existence of specific ganglioside-receptors in immobilized rat brain membranes has been proposed (40).

In the context of neural repair, the interaction of gangliosides with trophic factor receptors could explain the activation or inhibition of trophic responses. In this regard, it is worth noting that clustering of surface GM_1 by the β-subunit of cholera toxin can induce proliferation of thymocytes (33) or bi-modal (stimulatory or inhibitory) responses in the 3T3 fibroblast cell line (32), depending upon the state of growth.

Whether gangliosides inserted into the plasma membrane can themselves act as "receptors" for neurotrophic factors remains to be determined. Alternatively, the possibility remains that endogenous as well as exogenous gangliosides (and glycosphingolipids in general) might modulate receptor states. The density and type of membrane gangliosides could influence membrane proteins such as trophic factor receptors (affinity, clustering or internalization). In this regard, Cheresh and collaborators utilizing immunogold to detect gangliosides have shown that the accumulation of GD2 ganglioside near the vitronectin receptor can alter the receptor's state (1, 2).

Whatever the mechanisms of action of these NGF-ganglioside interactions in the *in vivo* repair of CNS cholinergic neurons, they provide a pharmacological possibility to enhance restorative responses in the injured nervous system. In this lesion model, in addition to the previously discussed biochemical effects, ganglioside treatment also enhances the K^+ induced release of endogenous acetylcholine *in vivo* (24, 25) and improves the behavioural performance of cortically lesioned animals in passive avoidance and Morris water maze tasks (9). These aforementioned points are of potential clinical relevance in the quest to improve cortical cholinergic function in degenerative diseases of the central nervous system.

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ABBREVIATIONS

ChAT choline acetyltransferase
GM_1 ganglioside GM_1
HACU high affinity choline uptake
i.c.v. intracerebroventricular
i.p. intra peritoneal(ly)
NBM nucleus basalis magnocellularis
NGF nerve growth factor

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