

LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN THE RAT: MORPHOLOGICAL, BIOCHEMICAL AND BEHAVIORAL REPARATIVE EFFECT OF NERVE GROWTH FACTOR AND GANGLIOSIDE GM₁

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Abstract. Three- and fifteen-month old rats with a unilateral ibotenic acid lesion of the nucleus basalis magnocellularis (NBM) were used. In 3-month old rats, 4 days after the lesion a 34 and 33% decrease in high affinity choline uptake (HACU) rate was found in the ipsilateral frontal and parietal cortices, respectively. Twenty-one days later the lesioned rats showed a loss in the NBM choline acetyltransferase (ChAT)-positive cells, a marked decrease in ipsilateral cortical ChAT activity and an impairment of the acquisition of a passive avoidance conditioned response. If the lesioned rats received nerve growth factor (NGF) (10 µg i.c.v.) twice a week or daily administration of ganglioside GM₁ (GM₁) (30 mg/kg i.p.), beginning immediately after surgery, the decreases in the HACU rate and ChAT activity were significantly smaller and the behavioral performance was normal. A potentiation by GM₁ of NGF effects on the cholinergic neurons of the NBM occurred since no differences were detected between sham-operated rats and rats treated with NGF plus either the active (30 mg/kg) or inactive (10 mg/kg) dose of GM₁. The loss in the number of NBM ChAT-positive neurons was reduced by GM₁ or prevented by NGF administrations, indicating that the

two drugs prevent the cholinergic deficit by protecting the cholinergic neurons of the NBM from ibotenic acid neurotoxicity. GM₁ had no effect on ChAT activity decrease and behavioral impairment in 15-month old rats. The latter finding indicates an age-related loss of the ability of GM₁ to enhance neurotrophic activity in the NBM.

INTRODUCTION

Major cortical cholinergic innervation in the mammalian brain is provided by the large neurons of the NBM (10, 27). Electrolytic or neurotoxic lesions of the NBM in rats bring about a 50% decrease, approximately, in cortical ChAT activity, HACU rate, acetylcholine (ACh) release and turnover in the frontal and parietal cortices (5, 19, 34). The destruction of NBM neurons results in electrocorticogram changes with an increase in cortical delta wave activity (3, 23) and behavioral impairment. The latter includes deficits in the acquisition of passive and active avoidance responses (2, 5, 22, 30), T-maze, water maze and spatial task performance (19, 29, 45).

After unilateral NBM lesions an ipsilateral spontaneous recovery occurs within 20 days in the cortical HACU rate (33). The decrease in ChAT activity and behavioral impairment induced by a unilateral lesion of the NBM also undergo spontaneous recovery and within 6 months no significant differences can be detected between lesioned and sham-operated rats (5, 44). Both the HACU rate and ChAT activity recovery can be facilitated by repeated GM₁ (4, 33) and NGF (8, 16) administrations.

NGF promotes the development of mammalian forebrain cholinergic neurons and is necessary for maintenance of normal function during adult life (42, 46). It increases ChAT activity in cultures of fetal septal cholinergic neurons (18) and in the striatum of neonatal rats (29). NGF also raises ChAT levels in the hippocampus and prevents cholinergic medial-septal neuron degeneration following fimbria-fornix transection (17, 21) and age-associated atrophy of the cholinergic neuron of the NBM (11).

The biochemical, morphological and behavioral ameliorative effects of gangliosides after various types of brain insults have been attributed to an increase in survival of specific neuronal populations (1, 7, 40). It has been suggested that the effect of gangliosides on central nervous system lesions may depend on an enhancement of neurotrophic activity present in the damaged tissue (14, 41). Furthermore, *in vitro* and *in vivo* findings support an interaction between NGF and gangliosides. NGF-induced sprouting from dorsal root ganglia is inhibited by antiganglioside antibodies (36). A cooperative effect between NGF and GM₁ has been shown on neurite formation in PC12 pheochromocytoma cells (26)

and in preventing biochemical and morphological changes of NBM neurons after ischemic lesions of the cortex (6).

The aim of the present work was to determine whether interactions between NGF and GM₁ may occur *in vivo* on cortical cholinergic mechanisms following unilateral neurotoxic lesion of the NBM.

METHODS

Male Wistar rats (Charles River), 3 and 15 months old, were used.

Surgical procedure and drug treatment

Under ketamine anesthesia (150 mg/kg) unilateral lesions of the NBM were made by injecting 25 nmol ibotenic acid in 0.5 μ l of 50 mM sodium phosphate-buffer (pH 7.5) with a 10 μ l Hamilton syringe. The injection lasted 3 min and the syringe was left in place for 5 min after completion of the infusion. The following coordinates, taken from the atlas for rat brain of Paxinos and Watson (32), were used: AP, 0.5 mm posterior to bregma, L, 2.8 mm lateral, H, 6.8 mm below the dura. In the sham-operated rats the syringe needle was lowered into the cortex and no ibotenic acid was injected. The histological examination of 10 μ m coronal sections through the lesion sites revealed that the lesions were located in the ventromedial region of the globus pallidus and that the cells of NBM were replaced by intense gliosis.

In the rats receiving NGF a polyethylene cannula (P.E. 10 tubing) was implanted into the lateral ventricle of the lesioned hemisphere and held in place by dental acrylic cement. 10 μ g of beta NGF (2.5 S) dissolved in 10 μ l of saline were administered through the cannula. The ganglioside GM₁ was dissolved in a sodium-phosphate buffer and was injected intraperitoneally (i.p.) daily in a volume never exceeding 0.5 ml. NGF from submaxillary glands and monosialoganglioside GM₁ were generously supplied by Fidia Res. Laboratories, Abano Terme, Italy.

Biochemical analysis

HACU rate and ChAT activity were measured 4 and 21 days after the operation, respectively.

HACU determination was performed according to the method of Simon et al. (38). (Methyl-³H) choline chloride (8.3 Ci/mol, Radiochemical Centre, Amersham) was used as label. Total high-affinity uptake was estimated and the uptake rates were corrected for filter retention of the label. The rates of choline uptake were expressed as pmol of choline in 4 min incubation time per mg protein.

ChAT activity was estimated by measuring the conversion of ¹⁴C acetyl-CoA (Radiochemical Centre, Amersham, spec. activity 59 mCi/

/mmol) to ^{14}C acetylcholine according to the method of Fonnum (12). Time incubation was 15 min at 37°C . ChAT activity was expressed as $\mu\text{mol/h}/100\text{ mg protein}$.

The protein content of the homogenates was measured according to the method of Lowry et al. (24).

Morphological analysis

Twenty-one days after lesioning, under deep pentobarbital anesthesia, the rats were perfused transcardially with Tyrode's solution, followed by a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4. The brains were then removed, postfixed for 3-5 h at room temperature (r.t.) and immersed in PBS overnight at 4°C . The day after, NBM coronal sections of $40\ \mu\text{m}$ were made and processed for counting positive ChAT cells. The sections were incubated free-floating for 12 h with an anti-ChAT monoclonal antibody from rat-mouse hybridoma cells (Boehringer, Type 1) at a concentration of $2.5\ \mu\text{g/ml}$. After several washes in PBS the sections were incubated for 1 h at r.t. in biotinylated goat anti-rat serum, washed again in PBS and incubated for 1 h in avidin-biotin-glucose oxidase complex. The glucose-oxidase label was revealed by using tetrazolium salts (TNBT, vector) in 50 mM Tris (pH 8.2) for 20 min in the dark. ChAT-positive profiles were counted at $10\times$ objective and analyzed by means of a computerized image analysis system (IBAS, Contron, Zeiss) coupled to a Zeiss photomicroscope.

Behavioral test

Twenty days after lesioning all rats were trained in a two-compartment step through passive avoidance apparatus (5). In the training trial, each rat was placed in the illuminated compartment and the latency between the door opening and entrance into the dark compartment was measured. When the rat placed all 4 paws in the dark chamber, a 1.5 mA scrambled shock was delivered to the grid floor for 5 s. The trial was terminated when the rat ran back into the illuminated compartment. Twenty-four hours after training, the latency between door opening and entrance in the dark compartment was recorded. The rat was allowed to remain up to 120 s in the illuminated compartment without walking into the dark chamber before being removed.

RESULTS

High-affinity choline uptake

The differences in HACU rate in the frontal (A) and parietal (B) cortices, expressed as percent changes from sham-operated rats, are shown

in Fig. 1. In 3-month old sham-operated rats the HACU rate in frontal and parietal cortices was 2.51 ± 0.03 and 2.41 ± 0.08 pmol/4 min/mg protein, respectively, while in saline treated lesioned rats it was 1.66 ± 0.09 and 1.62 ± 0.13 . If the lesioned rats received either a single i.c.v. administration of $10 \mu\text{g}$ NGF immediately after the lesion or 4 daily i.p. injections of $30 \mu\text{g}/\text{kg}$ GM₁, the decrease in HACU rate was about half as large as in the saline-treated rats. No differences in HACU rate in frontal and parietal cortices were observed between sham-operated rats and lesioned rats receiving a single i.c.v. administration of NGF plus 4 i.p. injections of $10 \text{ mg}/\text{kg}$ GM₁. This dose given alone was inactive (data not shown).

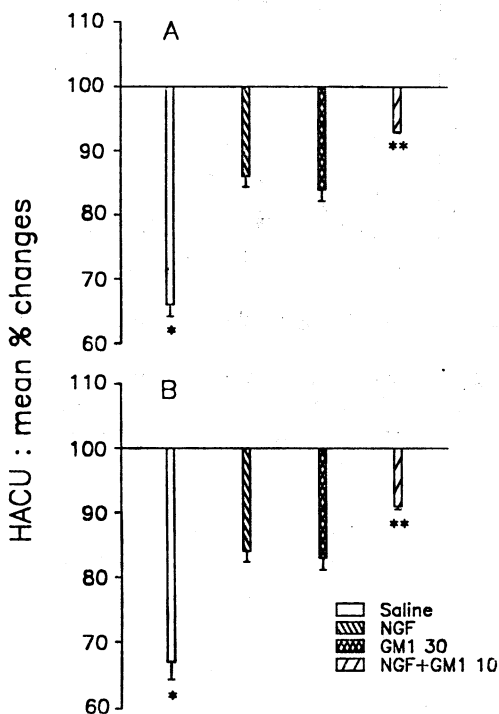


Fig. 1. Effects on NGF ($10 \mu\text{g}$, i.c.v.), GM₁ ($30 \text{ mg}/\text{kg}$, i.p.) and NGF plus GM₁ ($10 \text{ mg}/\text{kg}$ i.p.) on high affinity choline uptake in frontal (A) and parietal (B) cortices of rats with unilateral lesions of the NBM. Each column is the mean for 5-7 rats and indicates the mean percent changes from sham operated rats. Vertical bars = SEM. Student's two-tailed *t*-test: **p* < 0.001 vs. sham-operated rats; ***p* < 0.01 vs. saline-treated lesioned rats.

Choline acetyltransferase

The changes in ChAT activity induced by a unilateral lesion of the NBM and drug treatments in 3-month old rats are shown in Fig. 2. In

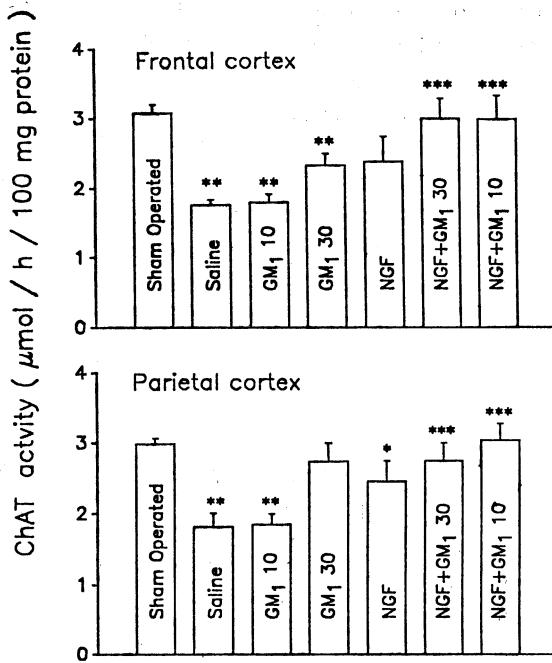


Fig. 2. Effect of GM₁ (10 and 30 mg/kg day for 21 days, i.p.), NGF (10 μg, twice a week for 3 weeks, i.c.v.) and NGF plus GM₁ (10 or 30 mg/kg/day) on ChAT activity in frontal (upper panel) and parietal (lower panel) cortices of rats with unilateral lesion of the NBM. Each column is the mean for 5-7 rats. Student's two-tailed t-test: **p* < 0.05, ***p* < 0.01 vs. sham-operated rats; ****p* < 0.01 vs. saline-treated lesioned rats.

sham operated rats ChAT activity in the frontal (upper panel) and parietal cortex (lower panel) was 3.08 ± 0.12 and 2.99 ± 0.08 μmol/h/100 mg protein, respectively. In lesioned rats receiving daily i.p. injections of saline a significant decrease in cortical ChAT activity occurred 21 days after surgery as compared to sham operated rats. The decrease was 43% and 39% in the frontal and parietal cortex, respectively. A similar reduction was found in lesioned rats injected i.p. daily for 21 days with 10 mg/kg GM₁ starting immediately after the lesion. A smaller decrease of ChAT activity occurred in lesioned rats treated either i.p. daily with 30 mg/kg GM₁, or i.c.v. twice a week for three weeks with 10 μg NGF. Normal values of ChAT activity were observed in lesioned rats receiving both 10 μg/kg NGF i.c.v. twice a week for three weeks and 10 or 30 mg/kg i.p. GM₁ daily for 21 days.

NGF and NGF plus the largest dose of GM₁ also brought about a slight but statistically significant increase in ChAT activity in the contralateral unlesioned hemisphere (data not shown).

If the lesion was made in 15-month old rats the decrease in ChAT activity in saline-treated rats 21 days after operation was 49% and 43% in the ipsilateral frontal and parietal cortex, respectively (Table I). In these rats daily i.p. injections for 21 day of 30 mg/kg GM₁ did not reduce the decrease of cortical ChAT activity caused by the NBM lesion.

TABLE I

Effect of GM₁ treatment (21 days) on ChAT activity ($\mu\text{mol/h}/100\text{ mg protein}$) in the cerebral cortex of 15-month old rats with unilateral lesion of the NBM

Condition	Treatment	Dose of GM ₁ mg/kg	Cortical Area	
			Frontal	Parietal
Sham operated (5)	saline		2.96 \pm 0.24	2.96 \pm 0.30
Lesioned (5)	saline		1.50 \pm 0.10* (-49%)	1.69 \pm 0.24** (-43%)
Lesioned (5)	GM ₁	30	1.87 \pm 0.25**	1.66 \pm 0.11**

Student's two-tailed *t*-test: **p* < 0.05; ** *p* < 0.01 as compared to sham-operated values.

Cell counting

Figure 3 shows the differences in the number of ChAT immunoreactive profiles in the NBM of 3-month old lesioned rats expressed as per-

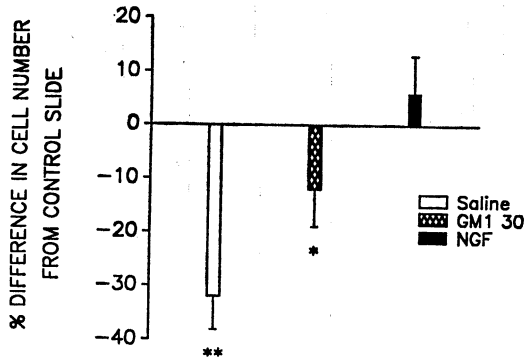


Fig. 3. Effect of GM₁ (30 mg/kg/day, i.p.) and NGF (10 μg , twice a week, i.c.v.) on the number of ChAT-positive neurons in the nucleus basalis of the lesioned rats. Each column is the mean for 4-6 rats and indicates the mean percent difference from the corresponding area of the contralateral hemisphere. Vertical bar = SEM. Statistical analysis: one-way analysis of variance (ANOVA test) followed by Tukey's test to determine differences among the means. ***p* < 0.01;

**p* < 0.05.

cent changes from the contralateral unlesioned side. Twenty-one days after surgery, a 32% decrease in the number of positive ChAT neurons was detected in the NBM of lesioned rats treated with saline. The loss was only 12% in lesioned rats administered i.p. with 30 mg/kg/day of GM₁. There was no difference in positive ChAT neurons between lesioned and unlesioned NBM in rats receiving 10 µg NGF twice a week for 3 weeks i.c.v.

Passive avoidance conditioned responses

The effects of the NBM lesion and drug treatment on the acquisition and retention of a passive avoidance conditioned response in 3-month old rats are shown in Fig. 4. The rats with a unilateral lesion receiving a learning trial 20 days after surgery and retested 24 h later showed a marked impairment in the passive avoidance conditioned response, as shown by shorter retest latencies. On the contrary, no significant impairment was detected in lesioned rats treated with GM₁ (i.p., 30 mg/kg/day for 21 days), NGF (10 µg i.c.v. twice a week for 3 weeks) and NGF plus GM₁ (i.p., 10 mg/day). Twenty-one days after the NBM lesion in 15-month old rats (Table II) the retest latencies were significantly shorter in saline treated rats and in rats receiving 30 mg/kg/day i.p. of GM₁ for 21 days, compared to sham operated rats.

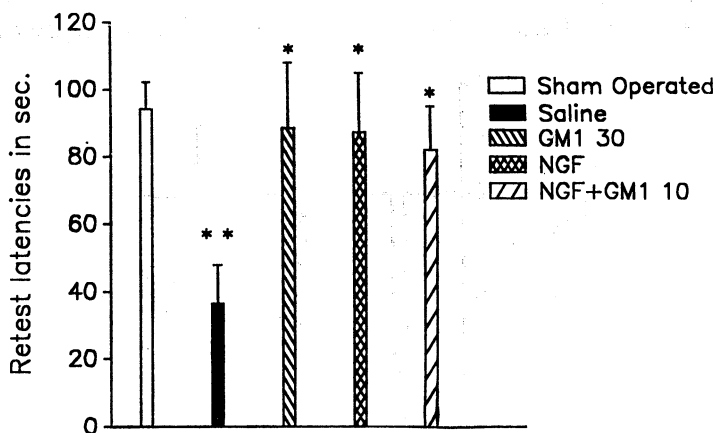


Fig. 4. Effect of GM₁ (30 mg/kg/day, i.p.), NGF (10 µg, twice a week, i.c.v.) and NGF plus GM₁ (10 mg/kg/day, i.p.) on the acquisition of a passive avoidance conditioned response 21 days after unilateral lesion of the NBM. Each column is the mean for 6-10 rats. Retest latencies are expressed in second (mean + SEM). Student's two-tailed *t*-test **p* < 0.05 vs. saline-treated lesioned rats; ***p* < 0.001 vs. sham-operated rats.

TABLE II

Effect of GM₁ (21 days) on passive avoidance conditioned response in 15-month old rats with unilateral lesion of the NBM

	Treatment mg/kg i.p.	Retest latencies s (Mean ± SEM)
Sham-operated (8)	—	59.4 ± 10.5
Lesioned (5)	saline	13.4 ± 7.6*
Lesioned (5)	GM ₁ 30	14.6 ± 12.2

* $p < 0.01$, statistically significant difference from sham-operated rats (ANOVA test).

DISCUSSION

A unilateral ibotenic acid lesion of the NBM results in a loss of the ChAT positive cells associated with a marked decrease in ipsilateral cortical ChAT activity, HACU rate, and behavioral impairment. In 3-month old lesioned rats, the treatment with NGF or GM₁ alone reduces the decrease in both HACU rate and ChAT activity. Conversely, the concomitant administration of NGF and GM₁ completely prevents the decrease in ChAT activity and the HACU rate. Furthermore, our experiments show that a complete biochemical recovery occurred not only when NGF was concomitantly administered with the largest dose of GM₁, which by itself is active, but also with the low inactive dose of GM₁. The latter results demonstrate that GM₁ potentiates NGF effects. The potentiation of NGF effects by GM₁ has been demonstrated *in vitro* on neurite formation in PC 12 cells (9), and chicken embryonic sensory neurons (39). *In vivo* NGF and GM₁ act synergistically to prevent the biochemical and morphological changes of rat basal forebrain neurons after ischemic lesions of the cortex (6).

The lesion-induced loss of ChAT cells in the NBM was attenuated by GM₁ and completely prevented by NGF treatment. Since drug treatments began immediately after lesioning, protection from neuronal damage and death rather than recovery could be the reason for the attenuation of the biochemical deficit and the better performance of passive avoidance conditioned response observed in the treated rats. Similarly, i.c.v. administration of NGF attenuates ChAT decrease and memory deficits following fimbria-fornix transection and prevents axotomy-induced cell death in the septum (17, 47). GM₁ has been shown to protect

the neurons from a variety of toxic and neurochemical insults and to facilitate neuronal regeneration (43). The possible mechanisms through which GM₁ protects neuronal membranes and functions from damage have been recently reviewed (25). The highest NGF levels in brain tissue appear within the target areas of the cholinceptive basal forebrain system (35). Radiolabeled NGF injected into target regions is taken up and retrogradely accumulated by the cholinergic neurons innervating them, such as the medial septal neurons for the hippocampus and NBM neurons for the cortex (13, 37). NGF exerts its effect after binding to specific receptors (46) and triggers a still unclear chain of events (15) leading to prevention of neuronal death and/or an enhancement of repair mechanisms. GM₁, in our experimental condition, may potentiate NGF effect either by acting at a receptor level or on the events following receptor activation.

It may be assumed that the lack of effect of GM₁ on ChAT decrease and behavioral impairment in 15-month old lesioned rats depends on a substantial reduction of NGF available and/or in a loss of its receptors in the tissue. A loss of NGF receptor-immunoreactive cells in the basal forebrain has been reported in 30-month old rats (20) and the supply of trophic factors in response to brain injury is reduced with age (31). If an altered NGF receptor expression already occurs in 15-month old rats, it may reduce the ability of GM₁ to enhance neurotrophic activity present in the damage tissue and a higher dose of GM₁ might be required to induce recovery.

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