

## Expression of astroglial nerve growth factor in damaged brain

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**Abstract.** This article provides an overview of different brain insults leading to induction of astroglial NGF expression. It discusses mechanisms of induction of astroglial NGF in a damaged brain and a possible role of its expression in pathophysiology of Alzheimer's disease. It emphasizes that the pharmacological manipulation of astroglial production of NGF may serve as a possible therapeutic strategy to ameliorate the pathological changes occurring after brain damage.

**Key words:** brain damage (surgical, neurotoxic, ischemic), NGF, astrocytes, Alzheimer's disease

## INTRODUCTION

Nerve growth factor is a proposed therapeutic agent for the treatment of neurodegenerative disorders such as Alzheimer's disease based on its ability to rescue and maintain cholinergic neurones in animal models of neurodegeneration and to enhance memory in models of induced cognitive defects (see Hefti and Weiner 1986). In fact, Olson et al. 1992, have recently described the initial positive results of direct infusion of NGF into Alzheimer's patient.

Although previous knowledge concerning NGF effects was limited to survival of cholinergic basal forebrain neurones, it has been recently found that NGF protects also against delayed neuronal death which develops in CA1 subfield of the hippocampus several days after global ischemia in an animal model of human cerebrovascular pathophysiology (Shigeno et al. 1991). Some recent data also point to a protective role of NGF against hypoglycemia induced changes in the hippocampus (Cheng and Matson 1992). Recent findings (Kerwin et al. 1991) demonstrated NGF receptors in the hippocampus which may provide an explanation of why NGF may act also in this structure. The mechanisms underlying the effects of NGF appear to be diverse possibly comprising receptor interactions, transport processes, protection against suicidal program leading to neuronal death etc (see references e.g. Shigeno et al. 1991, Crutcher et al. 1993).

NGF does not cross the blood brain barrier, due to its physicochemical properties. Thus, a direct administration remains so far the only means of its delivery.

As an alternative to this invasive, neurosurgical procedure, a more practical approach for the treatment of neurodegeneration may be to find compounds that would penetrate the blood brain barrier and mimic the action of NGF (see e.g. Friden et al. 1993).

Another feasible alternative for the treatment of neurodegeneration may be to influence pharmacologically the expression of endogenous levels of NGF.

Controversy still surrounds the cellular source of endogenous NGF in the brain. Apparently conflicting results have been obtained from *in vitro* and

*in vivo* studies. While it is clearly established that *in vitro* astrocytes synthesize and secrete NGF (see e.g. Furokawa et al. 1987), *in vivo* studies indicate neuronal localization and synthesis of NGF in target neurones (see e.g. Ayer-LeLievre et al. 1988, Bacia et al. 1992).

Several *in vivo* studies indicate that NGF from target neurones in the hippocampus and cortex, where it is synthesized, is retrogradely transported to the perikarya of cholinergic basal forebrain neurones and thus that the maintenance of the latter depends on constant trophic supply from the target neurones. These findings were crucial for formulation of the trophic hypothesis (see Hefti and Weiner 1986).

Recently, however, some data (Sofroniew et al. 1990, Kordower et al. 1992) questioned the trophic hypothesis in its original form. It has been demonstrated that excitotoxic lesions of the hippocampus, which destroy most endogenous NGF producing hippocampal neurones, do not lead to a decrease in viability of septal cholinergic neurones. This is in contrast to the pattern of degeneration seen following fimbria - fornix transection which injures the cell in addition to physically disconnecting it from its hippocampal target. The results obtained following excitotoxic lesions are furthering the notion that some local factors within the septal region, after disconnecting it from the target source, may play a role in maintenance of the cholinergic neurones.

Increasing evidence is recently accumulating that, although in intact brain the main source of NGF are target neuronal cells, in some pathological conditions an increase in extraneuronal pool of NGF takes place. Several lines of evidence indicate (see below) that in *noxious* conditions astrocytes may behave just as the astroglia *in vitro* and thus may be the site of NGF production in damaged brain. This raises hope that a successful pharmacological intervention may be focused on astrocytes, the "spared" if not meaningfully activated cell type of the damaged brain. To this point understanding the mechanisms involved in expression of astroglial NGF is necessary to further exploit them to pharmacologically increase endogenous NGF expression.

## **INDUCTION OF NERVE GROWTH FACTOR IN BRAIN FOLLOWING SURGICAL, NEUROTOXIC AND ISCHEMIC INSULTS**

The rise of NGF levels in brain in various models of reactive gliosis has indirectly shown that brain astrocytes may synthesize the nerve growth factor. Moreover, in several cases NGF immunoreactivity has been directly revealed in activated astrocytes.

Earlier studies (Weskamp et al. 1986, Lorez et al. 1988) revealed that transection of septohippocampal connections and the removal of cholinergic nerve terminals of the basocortical system, i.e. the deprivation of the cholinergic neurones of trophic support from the target, resulted in a long-lasting increase of NGF in the septum and basal nucleus, respectively. A very intense increase in GFAP (an astrocytic marker) immunostaining has been observed in the source of innervation (septum) after interruption its connections with the target (Gage et al. 1988).

Previous studies (Korsching et al. 1985) reported an about 50% increase in hippocampal NGF following fimbria-fornix lesions and attributed this increase entirely to the inhibition of retrograde transport caused by destruction of basal forebrain afferents. However, in another lesion paradigm - neurotoxic damage caused by injection of quinolinic acid, when afferent axons and terminals were not destroyed, a large increase in total NGF content in the hippocampus was also observed (Bakhit et al. 1991). Moreover, pyramidal and granular layer neurones which produce NGF under normal conditions were lost and a large increase in GFAP immunoreactivity was seen instead.

Lorez et al. 1989 indicated that acute hypoxia increases NGF levels mostly in areas that contain inflammatory cells and reactive astrocytes.

An increase in global hippocampal NGF was observed in conditions of delayed neuronal death in CA1 subfield due to transient ischemia (Shigeno et al. 1991, Hashimoto et al. 1992, Shozuhara et al.

1992) when neuronal NGF in this region was in deficiency. At that time GFAP in individual astrocytes and the number of GFAP positive cells gradually increased throughout the hippocampus (Shozuhara et al. 1992). The authors suggest that reactive astrocytes replaced the level of neuronal NGF in CA1. However, in either study no direct proof was provided as to whether reactive astrocytes identified in the CA1 subfield produced NGF, since no staining for NGF immunoreactivity was performed.

Tanaka et al. (1992) found that reactive astrocytes appeared in CA1 before the onset of visible neuronal degeneration indicating that signals from affected neurones may be transmitted to astrocytes for their quick functioning. These observations also point to the possibility that astrocytes play a role in neuronal degeneration induced by ischemia and indicate NGF production by astrocytes following ischemia.

Using a double immunostaining for NGF and GFAP and applying a septo-hippocampal pathway lesion paradigm it has been found that 7 days after injury, both in the hippocampus and in septum astrogliosis is connected with the appearance of NGF immunoreactivity in astrocytes (Bacia et al. 1991, Oderfeld-Nowak et al. 1992). A colocalization of the two immunoreactive products has not been observed in intact brains (Bacia et al. 1992). A possibility cannot be excluded however, that the observed immunoreactivity in astrocytes is partly due to other NGF-like molecules (Oderfeld-Nowak et al. 1992). These data demonstrate NGF immunoreactivity in the situation when the target neurones in the hippocampus do not cease to produce this factor. Various staining patterns of NGF immunoreactivity in astrocytes were found in this study which perhaps represent various stages of astrocytic activation. One of the forms, a typical "halo"-reaction of NGF immunoreactivity around astrocyte, is illustrated in Fig.1. The observed phenomenon could also have suggested the binding of NGF produced by any other type of cells to some NGF receptors present on astrocytes whose expression could be upregulated by the lesion. However, recent



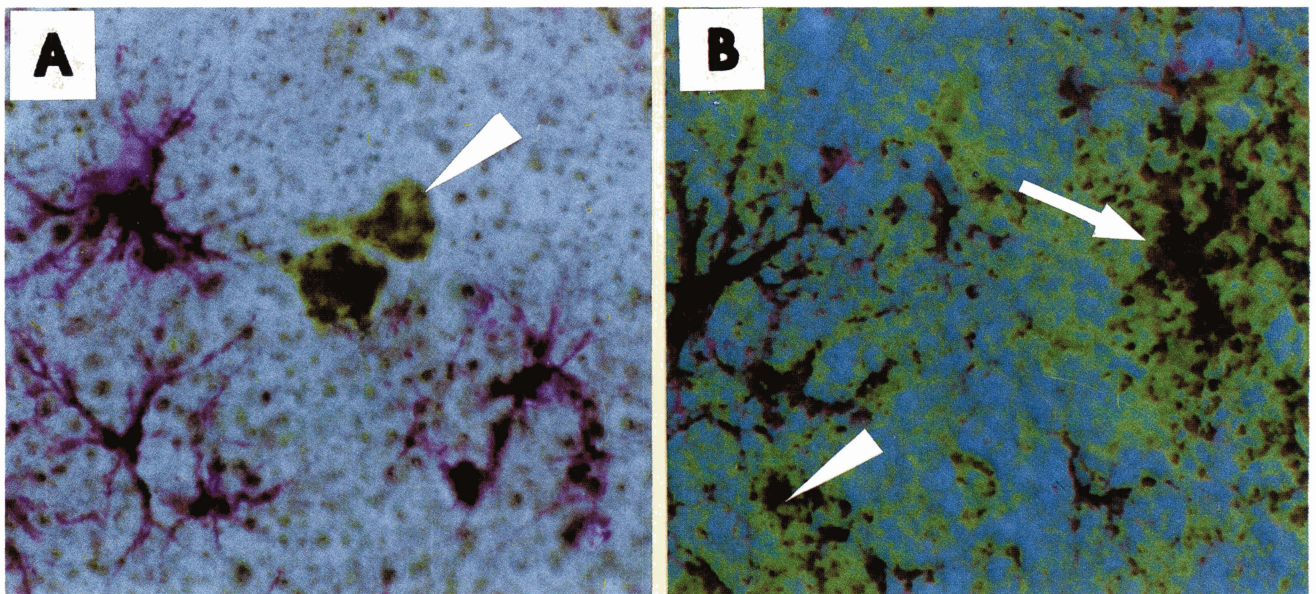


Fig.1. Photomicrographs of septal sections double-immunostained for NGF-IR (green) and GFAP-IR (dark blue) from: naive (A) and lesioned brain (B) exemplifying that in naive uninjured brain NGF-IR is connected with neurones (arrowhead), while in lesioned brain NGF-IR is also seen in many astrocytes (arrow) colocalizing with GFAP-IR. Photomicrographs were made with blue filter BG7. Magnification: (A, B) X 720.

data, showing that patterns of NGF-receptor immunoreactivity following injury do not correspond to patterns of NGF-IR, indicate that this is not the case (Bacia and Aloe, in preparation).

It is important to add, that not all astrocytes in both structures after the lesion of their connections revealed NGF immunoreactivity.

After a stab wound lesion of neostriatum, Altar et al. (1992) found that a total NGF content was highly elevated and, accompanying that, abundant, intensely labelled NGF immunoreactive cells with astrocytic-appearing processes were found around the lesion area. GFAP staining of adjacent sections was increased in comparison to control. In the injured neostriatum the density of NGF neuronal staining was reduced or absent, possibly because the NGF containing neurones were killed by mechanical trauma or because they failed to accumulate NGF. Thus, the data indicate that the increase in NGF content after stab injury occurs predominantly in GFAP positive astrocytes.

Destruction by local injection of quinolinic acid of the intrinsic hippocampal pyramidal and granule

cell layer neurones, resulted in a loss of neuronal NGF immunostaining and in intense NGF staining in some astrocyte-like cells in the lesioned area a week later (Bakhit et al. 1991). An increased NGF immunoreactivity in astrocytes correlated well with the degree of neuronal destruction.

In a recent study, Koczyk et al. (in preparation) found that exposure to trimethyltin, an environmental neurotoxin, also leads to an appearance of NGF immunoreactivity in astrocytes.

From the mentioned studies it was not possible to judge whether or not the activated NGF immunoreactive astrocytes were in the state of proliferation.

The presence of NGF immunoreactivity in activated astrocytes, in all above described pathological conditions, most likely indicate NGF synthesis by these astrocytes. However, it is not known whether the conditions of regulation of mRNA for NGF and expression of NGF protein are the same: a direct proof can only be provided by *in situ* hybridization studies performed on activated astrocytes. It has to be pointed out that *in situ*

hybridization with complementary probes for NGF have reported diminution of NGF hybridizing mRNA in hippocampal areas 7 days after destruction of neurones by kainic acid or colchicine (Ayer-LeLievre et al. 1988). Similarly, after ibotenic acid injection, Kordower et al. (1992), with RNA blot analysis revealed a decrease in NGF mRNA expression within the lesioned hippocampus. On the other hand a transient increase in mRNA for NGF in the granule cells of the hippocampus was reported following limbic seizures produced by electrolytic lesion (Gall and Isackson 1989), and shortly after kainic acid injection (Ballarin et al. 1991). Clearly, systematic investigations will be required to establish a temporal correlation between the expression of mRNA for NGF and that of the protein itself.

### **AN INCREASED LEVEL OF NGF-LIKE ACTIVITY IN ALZHEIMER'S DISEASE: DOES THIS INVOLVE EXPRESSION OF ASTROGLIAL NGF ?**

In postmortem tissue samples from patients with AD an increase in NGF-like activity was found. A weak cross reactivity with rBDNF and rNT-3 was noted in these studies with the bioassay and immunoblots, but not with ELISA assay (Crutcher et al. 1993). An increase was especially observed in cortex samples. These observations are in apparent conflict with the hypothesis of Hefti (Hefti and Weiner 1986) which suggested that a loss of trophic support (by NGF) in the cortex was linked with the degeneration of cholinergic markers in AD. In general, the data concerning NGF content changes in AD are somewhat contradictory (see references: Crutcher et al. 1993).

Interestingly, a decrease in BDNF mRNA in the hippocampus from AD patients with no change in NGF mRNA has recently been reported (Phillips et al. 1991).

The increase in NGF content, according to Crutcher et al. (1993), may represent a response to

degenerative changes. It would be of great importance to determine whether increases in NGF-like activity also occur in other degenerative diseases. Some indications that this is the case were earlier reported by Otten et al. (1989).

The cellular source of the increased NGF-like activity in AD is unknown. It is likely, however, that the neuropathological changes occurring in the cortex in AD patients may lead to increased glial production of NGF. This assumption is supported by recent findings concerning gliosis occurring with aging and, in degenerative diseases of the CNS, including AD (see Frederickson et al. 1992 for references). A very high (eight to sixteen fold) increase in GFAP content in all brain regions in AD patients compared to controls has been also reported.

If the increase in NGF-like activity in AD reflects an active response to atrophic changes in the basal forebrain, the rationale for enhancing it may be strengthened.

### **MECHANISMS OF INDUCTION OF ASTROGLIAL NERVE GROWTH FACTOR**

Little is known about the mechanisms regulating NGF production in astrocytes in brain in pathological conditions. Cytokines, especially interleukin-1 $\beta$ , have been postulated as endogenous regulators of NGF in both astrocytes and neurones (Otten et al. 1989, Bandtlow et al. 1990, Spranger et al. 1990, Oderfeld-Nowak et al. 1992). The increased level of cytokines in the brain in these conditions may be connected with inflammation and/or their production by injured brain elements themselves. The fact that IL-1 $\beta$  administration results in an increase in NGF levels (Otten et al. 1989, Spranger et al. 1990, Oderfeld-Nowak et al. 1993) and in appearance of NGF immunoreactive astrocytes (Bacia et al. 1991, Oderfeld-Nowak et al. 1992) in conjunction with a non additive effect on NGF content of combined lesion and IL-1 $\beta$  administration (Oderfeld-Nowak et al. 1993), speak in favour of the hypothesis that endogenous IL-1 $\beta$ , triggered by brain damage, is in-

volved in NGF induction. Moreover, (see references: Oderfeld-Nowak et al. 1992) the presence of IL-1 $\beta$  and its receptor in the brain, codistribution of IL-1 $\beta$  and NGF mRNA and stimulation by IL-1 $\beta$  of brain astrogliosis, strongly support this notion.

The sustained *in vivo* induction of NGF in astrocytes by IL-1 $\beta$  is consistent with its effects seen in primary culture of astrocytes (Carman-Krzan et al. 1991, Friedman et al. 1992). Long lasting NGF induction may be related to the relatively long-term cellular changes elicited by IL-1 $\beta$ . It is proposed that IL-1 $\beta$  increases NGF mRNA levels via both transcriptional activation and RNA stabilization and that these effects may be regulated via the arachidonic acid cascade (see references: Friedman et al. 1992).

Recent observations (see e.g. Zafra et al. 1990) have indicated that the NGF production in brain may be regulated via NMDA and non-NMDA receptors. The available data concern global and/or neuronal NGF. However, in contrast to neurones, NGF synthesis in astrocyte culture which produces substantial quantities of NGF protein is not regulated by neurotransmitters (Spranger et al. 1990). It is still to be verified whether such regulation of NGF in astrocytes may occur in pathological conditions *in vivo*.

We are not going here into more details concerning possible mechanisms of neuronal or astrocytic NGF induction as obtained in many experiments performed *in vitro*. Different substances including a variety of growth factors and cytokines may increase NGF and its mRNA expression by distinct mechanisms. For example, some works suggest that cAMP, protein kinase C and c-fos protein function as potential mediators of stimulated increases in NGF and NGF mRNA expression. These mechanisms remain to be identified. Messenger RNA NGF is suggested to be indirectly modulated through increasing intracellular Ca<sup>+2</sup> content. Increases in the latter, on the other hand, are known to increase immediate early genes including c-fos and c-jun. These immediate early gene products are postulated to enhance NGF expression e.g. in pharmacologically stimulated cultured astrocytes through inter-

action with a putative AP-1 site on the NGF promoter (for references see Friedman et al. 1992).

## CONCLUDING REMARKS

Although the role of astroglial NGF synthesized in pathological conditions in brain is still an enigma, it may be viewed as an response of glial cells to minimize neuronal damage and promote compensatory processes.

It is to be kept in mind that apart from NGF, also other neurotrophic factors including neurotrophins like BDNF or NT-3, may also be synthesized and released by astrocytes in damaged brain. Thus, astroglial NGF alone, or possibly in concert with other neurotrophic factors produced by astrocytes, may serve to ameliorate the pathological changes occurring in neurones.

In this respect astroglia may thus be the rational, albeit somewhat overlooked, target for drug intervention in cases of neurological diseases.

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## Abbreviations:

AD	Alzheimer's disease
BDNF	brain-derived neurotrophic factor
cAMP	cyclic adenosine monophosphate
DND	delayed neuronal death
ELISA	enzyme-linked immunosorbent assay
GFAP	glial fibrillary acidic protein
IL-1 $\beta$	interleukin-1 $\beta$
mRNA	messenger RNA
NGF	nerve growth factor
NGF-IR	NGF immunoreactivity
NMDA	N-Methyl-D-aspartate
NT-3	neurotrophin-3
rBDNF	recombinant BDNF
rNT-3	recombinant NT-3



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