

BRAIN REPAIR

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Nerve Growth Factor (NGF) in the Central Nervous System: Implications for the Treatment of Alzheimer's Disease

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INTRODUCTION

The physiological functions of NGF in the peripheral nervous system are already known for a long time (see Levi-Montalcini and Angeletti, 1968; Greene and Shooter, 1980; Thoenen and Barde, 1980). NGF regulates the regionally differential survival of sympathetic and neural crest-derived sensory neurons. This regulatory function comes into play when the axons of NGF-responsive neurons reach their target area (see Davies et al., 1987; Barde, 1989). Furthermore, NGF is essential for the differentiation and maintenance of neuron-specific functions such as the synthesis of enzymes involved in the production of neurotransmitters and neuron-specific peptides e.g. substance P and somatostatin (see Thoenen and Barde, 1980; Otten 1984). NGF acts as a retrograde messenger transferring information from the fields of projection in the periphery to the innervating NGF-responsive neurons (see Davies et al., 1987; Barde 1989). Following Darwinian principles, NGF is produced in the target fields in very small quantities permitting the survival of a limited number of neurons only. Injection of exogenous NGF increases the survival of NGF responsive neurons, thus abolishing natural cell death which occurs during normal development (see Hendry, 1980; Thoenen and Barde, 1980).

Although the physiological function of NGF in the peripheral nervous system is well documented some basic questions remain to be elucidated. For instance the nature of the signal transduction resulting from the interaction of NGF with its specific receptor is unknown as well as the regulatory mechanisms determining the time and location of NGF synthesis during

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development and in adulthood. For example, in the *vas deferens* the levels of NGF in the muscle layer which is densely innervated by sympathetic fibers are 20 times higher than in the sparsely innervated mucosa.

In contrast to the peripheral nervous system relatively little is known about the physiological functions of NGF in the central nervous system (CNS); the available information has appeared predominantly during the last few years. In the following report we will survey briefly the history of the detection and the spectrum of established actions of NGF in the CNS (see Thoenen et al., 1987a; Whittemore and Seiger, 1987) and delineate the possible links between the function of NGF in the brain and its potential therapeutic use for the treatment of Alzheimer's disease (see Hefti and Weiner, 1986).

For the treatment of Alzheimer's disease several approaches may be adopted. The local infusion of recombinant human NGF or the transplantation of "engineered" homologous or autologous cells secreting (augmented quantities of) human NGF seem to be the most straightforward procedures. Injection of peptides with NGF-like activity and therefrom deduced non-peptide molecules represent additional possibilities although the rationales for such approaches are not yet established. An attractive alternative to all these approaches is the modification of the synthesis of endogenous NGF. The understanding of the normal physiological regulation is the basis for a rational pharmacological modification. In this context we will compare the regulation of NGF synthesis in the periphery with that in the central nervous system; in particular we will focus on the role played by cytokines and lymphokines.

DETECTION OF SPECIFIC FUNCTIONS OF NGF IN THE CNS

In view of the fact that NGF plays a crucial role in the development and maintenance of specific functions of the peripheral sympathetic nervous system, the initial attempts to identify a physiological function of NGF in the CNS were directed towards the central adrenergic and dopaminergic neurons. The intraventricular and intracerebral injection of NGF did not result in any changes in tyrosine hydroxylase (TH) levels in these catecholaminergic systems. Conversely, the injection of anti-NGF antibodies did not reduce the TH levels (Fonkol et al., 1978; Schwab et al., 1979). In order to evaluate whether the absence of a response to NGF was

due to the absence of NGF receptors, labeled NGF was injected into the projection fields of the locus coeruleus. After injection of ^{125}I -NGF into the hippocampus there was no retrograde transport to the cell bodies of the locus coeruleus (they were retrogradely labeled by tetanus toxin and wheat germ agglutinin). However, unexpectedly, cell bodies of magnocellular cholinergic neurons of the septum were labeled (Schwab et al., 1979). Subsequent studies demonstrated that all cholinergic neurons of the basal forebrain nuclei, indeed, specifically transported retrogradely (Seiler and Schwab, 1984) and specifically bound in cryostat sections (Richardson et al., 1986; Raivich and Kreutzberg, 1987) ^{125}I -NGF. These neurons were also stained by anti-NGF-receptor antibodies and expressed the corresponding NGF-receptor mRNA. The same is true for the cholinergic interneurons in the striatum. The functionality of these NGF-receptors was then documented by an increase in ChAT levels after intraventricular injection of NGF in the early postnatal period (Ghahn et al., 1983; Mobley et al., 1985). In adult animals the response to injected NGF was only very small (Ghahn et al., 1983). However, after fimbria lesion NGF prevented the degeneration of the corresponding cholinergic neurons (Hefti, 1986). Springer and Loy (1985) impressively demonstrated that the absence of (Ghahn et al., 1983; Thoenen et al., 1987b) or the small effects (Vantini et al., 1988) of anti-NGF antibodies on ChAT levels in the brain results most probably from the very poor penetration of the antibodies. They showed that the reactive sprouting of perivascular sympathetic fibers into the hippocampus after fimbria lesion was prevented by the injection of anti-NGF antibodies exclusively in the immediate vicinity of the injection site. The evidence that NGF plays a physiological role in the CNS, in particular in the development and maintenance of function of the cholinergic neurons of the basal forebrain nuclei is also supported by the fact that the density of innervation by cholinergic fibers in the projection fields in the hippocampus, neocortex, and *bulbus olfactorius* corresponds with the levels of NGF and NGF-mRNA (Korsching et al., 1985; Whitemore et al., 1986; Large et al., 1986). Moreover, during development there is a close correlation between the increase in the levels of NGF and NGF-mRNA and those of ChAT in the hippocampus (Auburger et al., 1987).

In summary, the physiological importance of NGF for the cholinergic neurons of the basal forebrain nuclei and probably also for the cholinergic inter-

neurons in the striatum is strongly supported by experimental data. The functionality of other NGF-receptors identified by specific ^{125}I -NGF labeling in cryostat sections (Raivich et al., 1985; Richardson et al., 1986; Raivich and Kreutzberg, 1987), by staining with anti-NGF-antibodies (Yip and Johnson, 1987; Yan and Johnson, 1988; Sofroniew et al., 1989) or by *in situ* hybridization (Ernfors et al., 1988) remains to be established. In this context it is worth mentioning that cholinergic neurons of the brain stem neither express NGF-receptors nor do they respond to NGF. The functional significance of the transient expression of (low-affinity) NGF-receptors by motoneurons (Raivich et al., 1985; Yip and Johnson, 1987; Ernfors et al., 1988; Yan and Johnson, 1988) of the spinal cord and the specific retrograde transport of NGF after intramuscular injection (Yan et al., 1988) is not clear as a survival effect or an increase in ChAT levels has not been detected in motoneurons of chick or rat (Oppenheim et al., 1982; Yan et al., 1988).

RATIONALE FOR THE USE OF NGF IN THE TREATMENT OF ALZHEIMER'S DISEASE

The potential use of NGF in Alzheimer's disease is based on the observation that degenerative changes in the cholinergic neurons of the basal forebrain nuclei are a consistent finding (although not the only localization of degenerative changes) in early stages of Alzheimer's disease. This is also reflected by a decreased synthesis of acetylcholine in bioptic specimens of Alzheimer's patients. Moreover, there is agreement that the degenerative changes in cholinergic neurons of the basal forebrain nuclei are to a large extent responsible for the cognitive deficits in Alzheimer's disease (see Hefti and Weiner, 1986). These pathophysiological and clinical observations are complemented by experimental results which show that in rats the interruption of the ascending cholinergic projection from the basal forebrain nuclei results in cognitive deficits which can be restored by transplantation of fetal cholinergic neurons (Dunnett et al., 1982). In addition, cognitive deficits observed in subpopulations of aged rats correlate with the extent of atrophy and degeneration of the cholinergic neurons in the basal forebrain nuclei (Fischer et al., 1989). Most importantly, chronic administration of NGF over several weeks was found to improve both memory and learning capabilities and to increase the size of the cell bodies of

the cholinergic neurons in the basal forebrain (Fischer et al., 1987).

Alzheimer's disease does not seem to result from an insufficient production of NGF since no difference was found between the levels of NGF-mRNA in Alzheimer patients and aged-matched controls (Goedert et al., 1986). However, the fact that NGF has a protective or a curative effect after experimental damage (Hefti, 1986) or age-dependent atrophy of NGF-responsive neurons (Fischer et al., 1987) seems to represent a rational basis for the use of NGF in the treatment of Alzheimer's disease. Clearly, this approach provides only a limited symptomatic and not a causal treatment of the disease, since the degenerative changes are not restricted to the cholinergic neurons of the basal forebrain nuclei.

Since the transplantation of cholinergic neurons, the prolonged infusion of NGF after lesion or degenerative changes and the transplantation of engineered NGF secreting cells will be covered by other contributions to this Symposium, we will present the available information on the regulation of the synthesis of NGF in the central nervous system as compared to the periphery. The understanding of these mechanisms is the basis for a rational approach to the pharmacological regulation of NGF synthesis.

REGULATION OF NGF SYNTHESIS IN THE PERIPHERAL AND IN THE CENTRAL NERVOUS SYSTEM

In order to obtain information on mechanisms involved in the control of NGF synthesis in the peripheral nervous system we studied the (reactive) changes in NGF synthesis under experimental conditions of nerve degeneration and regeneration; it was hoped that mechanisms involved in such enhanced reactive synthesis might also have physiological relevance. After transection of the sciatic nerve a dramatic increase in the synthesis of NGF by the non-neuronal cells, mainly Schwann cells and fibroblasts, occurred (Heumann et al., 1987a,b). Under normal conditions these cells only insignificantly contribute to the NGF supply to responsive neurons whose axons run in the sciatic nerve. Moreover, *in situ* hybridization experiments demonstrated that all non-neuronal cells contributed to the enhanced NGF synthesis after lesion and not only those ensheathing axons of NGF-dependent neurons, i.e. postganglionic sympathetic and sensory neurons (Heumann et al., 1987a; Bandtlow et al., 1987). By comparing the time-

course of NGF-mRNA increase after lesion *in situ* with that in segments of sciatic nerve in culture it became apparent that the rapid initial increase was identical. However, the prolonged sustained increase observed *in vivo* was not detectable in culture (Heumann et al., 1987b). It was subsequently demonstrated that immigrating macrophages were responsible for this difference. When cultured segments of sciatic nerve were supplemented with activated macrophages or their conditioned medium the *in vivo* situation could be mimicked (Heumann et al., 1987b). The lymphokine Interleukin-1 (IL-1) was then demonstrated to be the agent predominantly responsible for the increased synthesis of NGF (Lindholm et al., 1987). Studying the effect of IL-1 in more detail, we found that IL-1 enhances both the transcription of NGF-mRNA and increases the stability of this message (Lindholm et al., 1988). In contrast to this stimulatory effect of IL-1 glucocorticoid hormones were found to down-regulate expression of NGF; dexamethasone, a synthetic glucocorticoid, given to rats prior to nerve transection virtually abolished the lesion-mediated increase in NGF-mRNA. In order to investigate at which levels glucocorticoids down-regulate NGF expression we have recently begun transfection experiments using the mouse NGF promoter linked to a chloramphenicol acetyltransferase (CAT) reported gene. Preliminary data indicate that dexamethasone directly inhibits the transcription of NGF-mRNA. Thus glucocorticoids may interfere with the synthesis of NGF after peripheral nerve lesion, a fact to be borne in mind when glucocorticoids are given after peripheral nerve lesion in order to reduce inflammatory reactions and the formation of oedema. This is of particular importance in view of the fact that the compensatory production of NGF by the non-neuronal cells of the sciatic nerve can only incompletely replace the interrupted supply of NGF from peripheral target sites. In the rat sciatic nerve, the compensatory production at the lesion site amounts only to about 40% of the normal supply from the periphery (Heumann et al., 1987a).

In comparison to the studies in the peripheral nervous system the investigations on the regulation of NGF synthesis in the CNS are, as mentioned above, far less advanced. In the periphery it has been demonstrated that a great variety of non-neuronal cells in the target areas of the NGF-responsive neurons are able to produce NGF; these include fibroblasts, smooth muscle cells, epithelia and Schwann cells (Bandtlow et al., 1987). In the CNS

only astrocytes have unambiguously been shown to synthesize NGF (Lindsay, 1979; Furukawa et al., 1986; 1987). However, more recently, *in situ* hybridization experiments provided evidence that NGF could be transcribed also in neurons of the rat hippocampus and neocortex (Rennert and Heinrich, 1986; Ayer-LeLièvre et al., 1988; Whittemore et al., 1988) indicating that in the CNS, in contrast to the periphery, neurons may contribute to the synthesis of NGF. The relative contribution of neurons and glial cells (astrocytes) to the synthesis of NGF in the target areas of NGF-responsive neurons remains to be established. In particular it has to be evaluated whether the release of NGF from neurons follows the regulated, secretory pathway or, as demonstrated for non-neuronal cells in the periphery (Barth et al., 1984), the constitutive non-regulated pathway.

In a first approach to study the physiological regulation of NGF synthesis in the brain we explored the effects of various growth factors on NGF-mRNA levels and NGF protein secretion into the medium of cultured rat astrocytes. The growth factors tested were those which have been shown immunologically or by *in situ* hybridization to be present in brain tissue and therefore potentially play a role in the physiological regulation of the synthesis of NGF. In order to substantiate the results obtained *in vitro* we compared the effects obtained in cell cultures with those seen *in vivo* after injection of the growth factors into rat brains. The results obtained are summarized in Table 1 and 2. They indicate that NGF-mRNA in cultured astrocytes can be influenced by various growth factors including IL-1. This lymphokine increased astrocyte NGF-mRNA levels about 5-7fold after 6 h of incubation. Besides IL-1, epidermal growth factor (EGF), transforming growth factor- α (TGF- α) and basic fibroblast growth factor (bFGF) elevated NGF-mRNA levels in astrocytes to a similar extent (see Table 1). After 24 h the NGF-mRNA levels approached control levels. This limited duration of the effects of the growth factors investigated was most probably due to their degradation or inactivation since re-addition of the same molecules to the culture medium after 24 h resulted in a re-increase comparable to that observed after the initial addition. By far the greatest effect on NGF-mRNA levels in astrocytes was observed with transforming growth factor- β (TGF- β); an approximately 20-fold increase was observed after the first 6 h of incubation. However, in contrast to the other growth factors investigated there was a further increase up to 24 h.

Table I. Increase in NGF-mRNA levels in cultured rat astrocytes by IL-1 and various growth factors.

| <u>Factor added</u> | <u>Fold increase (above controls) after</u> | |
|--|---|-------------|
| | <u>4 h</u> | <u>24 h</u> |
| Controls | 1 | 1 |
| Interleukin-1 β (0.8 ng ml ⁻¹) | 5 | 2 |
| Epidermal growth factor (5 ng ml ⁻¹) | 7 | 2 |
| Transforming growth factor- α (10 ng ml ⁻¹) | 10 | 2 |
| Fibroblast growth factor, basic (5 ng ml ⁻¹) | 7 | 3 |
| Transforming growth factor- β -1 (5 ng ml ⁻¹) | 20 | 50 |

Astrocytes were incubated with the growth factors for 4 and 24 hr. NGF-mRNA was determined by quantitative Northern blot analysis. The values given represent the mean of 3 to 8 independent experiments.

Table II. Effect of intracerebroventricular injection of growth factors on NGF-mRNA levels in rat hippocampus.

| <u>Factor injected</u> | <u>Fold increase (above controls) after</u> | |
|--|---|-------------|
| | <u>6 h</u> | <u>24 h</u> |
| PBS, vehicle | 1 | 1 |
| Interleukin-1 β (2 ng) | 2 | 1 |
| Interleukin-1 β (16 ng) | 4 | nd*) |
| Fibroblast growth factor, basic (5 ng) | 2 | 1 |
| Fibroblast growth factor, basic (25 ng) | 2,5 | nd*) |
| Transforming growth factor- α (20 ng) | 1 | nd*) |
| Transforming growth factor- β 1 (5 ng) | 3 | 4 |

*) not determined.

Growth factors were injected in a volume of 4 μ l into the lateral ventricle of 8-10 day old rats. The animals were killed after various periods of time and NGF-mRNA in the ipsilateral hippocampus was determined.

The increases in astrocyte NGF-mRNA observed after the addition of various growth factors were followed by a corresponding increase in NGF protein secreted into the culture media. Since we did not observe either in Northern blots or by *in situ* hybridization any expression of NGF-mRNA in cultured oligodendrocytes and microglia it seems justifiable to conclude in view of the observed effects in astrocyte cultures (> 90% pure) that these are, indeed, the only brain glial cells synthesizing NGF. Our cultures did not contain neurons, in particular neurons from the target areas of NGF-responsive neurons. Experiments are in progress designed to establish whether these neurons express NGF-mRNA also under cultured conditions, as suggested by *in situ* hybridization, and how the regulation of NGF synthesis in these neurons compares with that in astrocyte cultures.

Comparing the *in vitro* data with the effects observed after injection of the corresponding growth factors *in vivo* a different picture became apparent (compare Table I and II). Whereas IL-1 increased NGF-mRNA in rat hippocampus *in vivo* almost to the same extent (4-fold) as in cultured astrocytes the effects of FGF and TGF- β were smaller *in vivo* than *in vitro* and that of TGF- α was even completely absent *in vivo*. The reasons for the discrepancies between the effects observed *in vivo* and those observed in cultures of astrocytes may be multiform. For instance, they may reflect differences in tissue penetration, differences in proteolytic degradation or inactivation and differences in the number and/or affinity of the growth factor receptors between astrocytes in culture and those *in vivo*. We have some preliminary information indicating that IL-1 up-regulates its own expression in the hippocampus, this may represent a possible feedback regulation for the action of IL-1 in the brain. Indeed, IL-1 receptors seem to be expressed in the same regions of the hippocampus (Farrar et al., 1987a,b) where, as demonstrated by *in situ* hybridization, IL-1- β -mRNA is expressed (Bandtlow et al., in preparation). Furthermore, *in situ* hybridization experiments have shown that IL-1-mRNA is also expressed in other brain regions - most likely also in neurons - including the *bulbus olfactorius*, the granular layer of the cerebellum, the neocortex, in particular the frontal cortex, restricted areas of the hypothalamus. The *in situ* hybridization experiments performed with probes for IL-1 β and NGF are compatible with at least a partial co-localization in the *bulbus olfactorius*, hippocampus and cerebral cortex (Bandtlow et al., in

preparation). In this context the future elucidation of the mechanism of release of IL-1, a cytosolic molecule, which has been demonstrated to be released in an unconventional manner from macrophages (see Hazuda et al., 1988) will be of great interest in order to evaluate the function of IL-1 in the regulation of NGF synthesis under physiological conditions in the CNS.

In conclusion, the results obtained indicate that, like in the periphery, after lesion and possibly also under physiological conditions, IL-1 and TGF- β may play a role in the regulation of NGF synthesis in the CNS. Experiments are in progress aiming at an elucidation which other cells, in addition to astrocytes, are responsive to IL-1 and TGF- β and how these molecules themselves are regulated in the CNS as an integral part of their regulatory function in the synthesis of NGF in the CNS.

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REFERENCES

- Auburger, G., Heumann, R., Hellweg, R., Korsching, S., and Thoenen, H. (1987). Developmental changes of Nerve Growth Factor and its mRNA in the rat hippocampus: Comparison with choline acetyltransferase. *Dev. Biol.*, 120, 322-328.
- Ayer-LeLièvre, C., Olson, L., Ebendal, T., Seiger, Å., and Persson, H. (1988). Expression of the β -nerve growth factor gene in hippocampal neurons. *Science*, 240, 1339-1341.
- Bandtlow, C.E., Heumann, R., Schwab, M.E., and Thoenen, H. (1987). Cellular localization of nerve growth factor synthesis by *in situ* hybridization. *EMBO J.*, 6, 891-899.
- Barde, Y.-A. (1989). Trophic factors and neuronal survival. *Neuron*, 2, 1525-1534.
- Barth, E.-M., Korsching, S., and Thoenen, H. (1984). Regulation of Nerve Growth Factor synthesis and release in organ cultures of rat iris. *J. Cell Biol.*, 99, 839-843.
- Davies, A.M., Bandtlow, C., Heumann, R., Korsching, S., Rohrer, H., and Thoenen, H. (1987). The site and timing of nerve growth factor (NGF) synthesis in developing skin in relation to its innervation by

- sensory neurones and their expression of NGF receptors. *Nature*, 326, 353-363.
- Dunnett, S.B., Low, W.C., Iversen, S.D., Stenevi, U. and Björklund A. (1982). Septal transplants restore maze learning in rats with fornix-fimbria lesions. *Brain Res.*, 251, 335-348.
- Ernfors, P., Hallböök, F., Ebendal, T., Shooter, E.M., Radeke, M.J., Misko, T.P., and Persson, H. (1988). β -nerve growth factor receptor mRNA in the chick and rat. *Neuron*, 1, 983-996.
- Farrar, W.L., Hill, J.N., Harel-Bellan, A., and Vinocour, M. (1987a). The immune logical brain. *Immunol. Rev.*, 199, 361-378.
- Farrar, W.L., Kilian, P.L., Ruff, M.R., Hill, J.M., and Pert, C.B. (1987b). Visualization and characterization of interleukin-1 receptors in brain. *J. Immunol.*, 139, 459-463.
- Fischer, W., Wictorin, K., Björklund, A., Williams, L.R., Varon, S., and Gage, F.H. (1987). Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature*, 329, 65-68.
- Fischer, W., Gage, F.H., and Björklund, A. (1989). Degenerative changes in forebrain cholinergic nuclei correlate with cognitive impairments in aged rats. *Eur. J. Neurosci.*, 1, 34-45.
- Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K. (1986). Synthesis and secretion of nerve growth factors by mouse astroglial cells in culture. *Biochem. Biophys. Res. Commun.*, 136, 57-63.
- Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K. (1987). Synthesis/secretion of nerve growth factor is associated with cell growth in cultured mouse astroglial cells. *Biochem. Biophys. Res. Commun.*, 142, 395-402.
- Gnahn, H., Hefti, F., Heumann, R., Schwab, M., and Thoenen, H. (1983). NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal forebrain: Evidence for a physiological role of NGF in the brain? *Dev. Brain Res.*, 9, 45-52.
- Goedert, M., Fine, A., Hunt, S.P., and Ullrich, A. (1986). Nerve growth factor mRNA in peripheral and central rat tissues and in the human central nervous system: Lesion effects in the rat brain and levels in Alzheimer's disease. *Mol. Brain Res.*, 1, 85-92.
- Greene, L.A. and Shooter, E.M. (1980). The nerve growth factor: Biochemistry, synthesis, and mechanism of action. *Ann. Rev. Neurosci.*, 3, 353-402.
- Hazuda, D.J., Lee, J.C., and Young, P.R. (1988). The kinetics of interleukin 1 secretion from activated monocytes. *J. Biol. Chem.*, 17, 8473-8479.

- Hefti, F. (1986). Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.*, 6, 2155-2162.
- Hefti, F. and Weiner, W.J. (1986). Nerve growth factor and Alzheimer's disease. *Ann. Neurol.*, 20, 275-281.
- Hendry, J.A. (1980). Proteins of the nervous system. 2nd ed. Bradshaw, R.A. and Schneier D.M. (eds.), Raven Press, New York, pp. 183-211.
- Heumann, R., Korsching, S., Bandtlow, C., and Thoenen, H. (1987a). Changes of nerve growth factor synthesis in non-neuronal cells in response to sciatic nerve transection. *J. Cell Biol.*, 104, 1623-1631.
- Heumann, R., Lindholm, D., Bandtlow, C., Meyer, M., Radeke, M.J., Misko, T.P., Shooter, E., and Thoenen, H. (1987b). Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: Role of macrophages. *Proc. Natl. Acad. Sci. USA*, 84, 8735-8739.
- Konkol, R.J., Mailman, R.B., Bendeich, E.G., Garrison, A.M., Mueller, R.A., and Breese, G.R. (1978). Evaluation of the effects of nerve growth factor and anti-nerve growth factor on the development of central catecholaminergic neurons. *Brain Res.*, 144, 277-285.
- Korsching, S., Auburger, G., Heumann, R., Scott, J., and Thoenen, H. (1985). Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J.*, 4, 1389-1393.
- Large, T.H., Bodary, S.C., Clegg, D.O., Weskamp, G., Otten, U., and Reichardt, L.F. (1986). Nerve growth factor gene expression in the developing rat. *Science*, 234, 352-355.
- Levi-Montalcini, R. and Angeletti, P.U. (1968). Nerve growth factor. *Physiol. Rev.*, 48, 534-569.
- Lindholm, D., Heumann, R., Meyer, M., and Thoenen, H. (1987). Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature*, 330, 658-659.
- Lindholm, D., Heumann, R., Hengerer, B., and Thoenen, H. (1988). Interleukin 1 increases stability and transcription of mRNA encoding nerve growth factor in cultured rat fibroblasts. *J. Biol. Chem.*, 263, 16348-16351.
- Lindsay, R.M. (1979). Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurons. *Nature*, 282, 80-82.
- Mobley, W.C., Rutkowski, J.L., Tennekoon, G.I., Buchanan, K., and Johnston, M.V. (1985). Choline

- acetyltransferase in striatum of neonatal rats increased by nerve growth factor. *Science*, 229, 284-287.
- Oppenheim, R.W., Maderdrut, J.L., and Wells, D.J. (1982). Cell death of motoneurons in the chick embryo spinal cord. VI. Reduction of naturally occurring cell death in the thoracolumbar column of Terni by nerve growth factor. *J. comp. Neurol.*, 210, 174-189.
- Otten, U. (1984). Nerve growth factor and the peptidergic sensory neurons. *Trends Pharmacol.*, 7, 307-310.
- Raivich, G., Zimmermann, A., and Sutter, A. (1985). The spatial and temporal pattern of BDNF receptor expression in the developing chick embryo. *EMBO J.*, 4, 637-644.
- Raivich, G., and Kreutzberg, G.W. (1987). The localization and distribution of high affinity β -nerve growth factor binding sites in the central nervous system of the adult rat. A light microscopic autoradiographic study using [125 I] β -nerve growth factor. *Neuroscience*, 20, 23-36.
- Rennert, P.D. and Heinrich, G. (1986). Nerve growth factor mRNA in brain: Localization by *in situ* hybridization. *Biochem. Biophys. Res. Commun.*, 138, 813-
- Richardson, P.M., Verge Issa, V.M.K., and Riopelle, R.J. (1986). Distribution of neuronal receptors for nerve growth factor in the rat. *J. Neurosci.*, 6, 2312-2321.
- Schwab, M., Otten, U., Agid, Y., and Thoenen, H. (1979). Nerve growth factor (NGF) in the rat CNS: absence of specific retrograde axonal transport and tyrosine hydroxylase induction in locus coeruleus and substantia nigra. *Brain Res.*, 168, 473-483.
- Seiler, M. and Schwab, M.E. (1984). Specific retrograde transport of nerve growth factor (NGF) from neocortex to nucleus basalis in the rat. *Brain Res.*, 300, 33-39.
- Sofroniew, M.V., Isacson, O., and O'Brien, T.S. (1989). Nerve growth factor receptor immunoreactivity in the rat suprachiasmatic nucleus. *Brain Res.*, 476, 358-362.
- Springer J.E. and Loy, R. (1985). Intrahippocampal injections of antiserum to nerve growth factor inhibit sympathohippocampal sprouting. *Brain Res. Bull.*, 15, 629-634.
- Thoenen, H. and Barde, Y.-A. (1980). Physiology of nerve growth factor. *Physiol. Rev.*, 60, 1284-1335.
- Thoenen, H., Bandtlow, C., and Heumann, R. (1987a). The physiological function of nerve growth factor in the central nervous system: Comparison with the

- periphery. *Rev. Physiol. Biochem. Pharmacol.*, 109, 145-178.
- Thoenen, H., Auburger, G., Hellweg, R., Heumann, R., and Korsching, S. (1987b). Cholinergic innervation and levels of nerve growth factor and its mRNA in the central nervous system. In Cholinergic Mechanisms. Ellis Horwood, Chichester, 379-388.
- Vantini, G., Fusco, M., Schiavo, N., Cavicchioli, L., Leon, A., and Toffano, G. (1988). *In vivo* effects of nerve growth factor (NGF) and anti-NGF antibodies on cholinergic neurons of rat basal forebrain. Senile dementia. II. Int. Symp., Agnoli, A., Cohn, J., Lassen, N., and Mayeux, R. (eds.) John Libbey Eurotext, Paris, pp. 339-343.
- Whittemore, S.R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, Å., Strömberg, I., and Persson, H. (1986). Developmental and regional expression of β nerve growth factor messenger RNA and protein in the rat central nervous system. *Proc. Natl. Acad. Sci. USA.* 83, 817-821.
- Whittemore, S.R. and Seiger, Å. (1987). The expression localization and functional significance of beta-nerve growth factor in the central nervous system. *Brain Res. Rev.*, 12, 439-464.
- Whittemore, S.R., Friedman, P.L., Larhammar, D., Persson, H., Gonzales-Carvajal, M., and Holets, V.R. (1988). Rat β -nerve growth factor sequence and site of synthesis in the adult hippocampus. *J. Neurosci. Res.*, 20, 403-410.
- Yan, Q. and Johnson Jr., E.M. (1988). An immunohistochemical study of the nerve growth factor receptor in developing rats. *J. Neurosci.*, 8, 3481-3498.
- Yan, Q., Snider, W.D., Pinzone, J.J., and Johnson Jr., E.M. (1988). Retrograde transport of Nerve Growth Factor (NGF) in motoneurons of developing rats: Assessment of potential neurotrophic effects. *Neuron*, 1, 335-343.
- Yip, H.K. and Johnson, E.M. (1987). Nerve growth factor receptors in rat spinal cord: an autoradiographic and immunohistochemical study. *Neuroscience*, 22, 267-279.