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COMMENTARY

NERVE GROWTH FACTOR (NGF) RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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Nerve growth factor (NGF) is the prototype of what is presumed to be a large family of "neurotrophic factors" which mediate the ability of target tissue to sustain survival and function of their innervating neurons. It has long been observed that neurons deprived of a target tissue will atrophy and often die. This target dependence is particularly critical in the developing animal as inferred by the rapid and extensive neuronal degeneration following experimental removal of the target organ. During normal development, most neuronal populations undergo an extensive, naturally occurring neuronal death. Loss of neurons is typically about 50% of the original population. A primary mechanism mediating this normal, physiological "programmed cell death" again appears to be neuronal dependence on target tissue because the cell death can be accentuated by removal of target or be reduced by experimental addition of target tissue (for review, see Refs. 1 and 2).

The most generally considered mechanism by which target tissues exert this action on neurons is via the elaboration of neurotrophic factors upon which innervating neurons are dependent. The only neurotrophic factor that has been isolated and chemically characterized, and for which a physiological role is well established, is NGF. In fact, much of the experimental evidence for the "neurotrophic factor hypothesis" is derived from the study of the biological effects exerted by NGF. Attempts to isolate and characterize other neurotrophic factors have met with only limited success for a variety of reasons, primarily a paucity of preparatively feasible sources. This important enterprise continues to attract many investigators and, hopefully, work over the next several years will produce other molecules which can join NGF as established physiological neurotrophic factors.

Until a few years ago, NGF was considered solely as a target-derived factor which acted only on sympathetic neurons and neural crest-derived sensory neurons of the peripheral nervous system (PNS). No role for NGF (nor any adverse effects of NGF deprivation) has been shown for other peripheral neuronal types (parasympathetic, placodally derived sensory, enteric, or motor neurons). NGF is required for survival and function of sympathetic neurons throughout their entire lifespan. Sensory neurons appear to require NGF for *survival* only during development (for review, see Ref. 3). NGF is required, however, for normal function of at least some, if not all, sensory neurons in the adult animal. The general mechanism by which NGF acts on responsive neurons is shown diagrammatically in Fig. 1. Considerable evidence supports each step in this scheme. Messenger RNA encoding NGF is produced in peripheral tissues in quantities generally correlating with the density of sympathetic innervation of these tissues [4]. Similarly, the levels of the NGF protein molecule are consistent with mRNA levels and innervation densities [5]. The quantities of NGF in these tissues are very low; there is no evidence that NGF is sequestered in any secretory organelle or that NGF is released in response to any stimulation. Instead, NGF appears to be synthesized constitutively and constantly released, rather than stored. An obvious exception to this statement is the highly concentrated storage in male mouse salivary glands which serve as the most generally used preparative source of the molecule, although no physiological significance of this storage has been established. After release from the target, NGF binds to specific NGF receptors (see below) [6]. NGF [7] and the NGF receptor [8] are then internalized and retrogradely transported to the neuronal cell body. The molecular nature of the signal conveyed from the periphery to the somata, which promotes neuronal survival and the other effects ascribed to NGF, may be the NGF molecule alone, the NGF:NGF receptor complex, or perhaps some modified form of the receptor. Alternatively, the signal could be some unknown second messenger generated by the NGF:NGF receptor interaction at the nerve terminal that is subsequently retrogradely transported. If this is the case, then the transport of NGF and the NGF receptor may serve merely as a means of regulating NGF catabolism by the cell or as a mechanism to regulate NGF and NGF receptor density at terminal areas. In any event, the precise mechanism(s) by which NGF produces its survival promoting and other effects is not known. Therefore, even though the work of many laboratories has contributed to the understanding of the NGF dependence of peripheral neurons and the general scheme (Fig. 1) by which NGF exerts its effects on these neurons, many aspects of its mechanism of action are essentially unknown.

As mentioned above, NGF has long been con-

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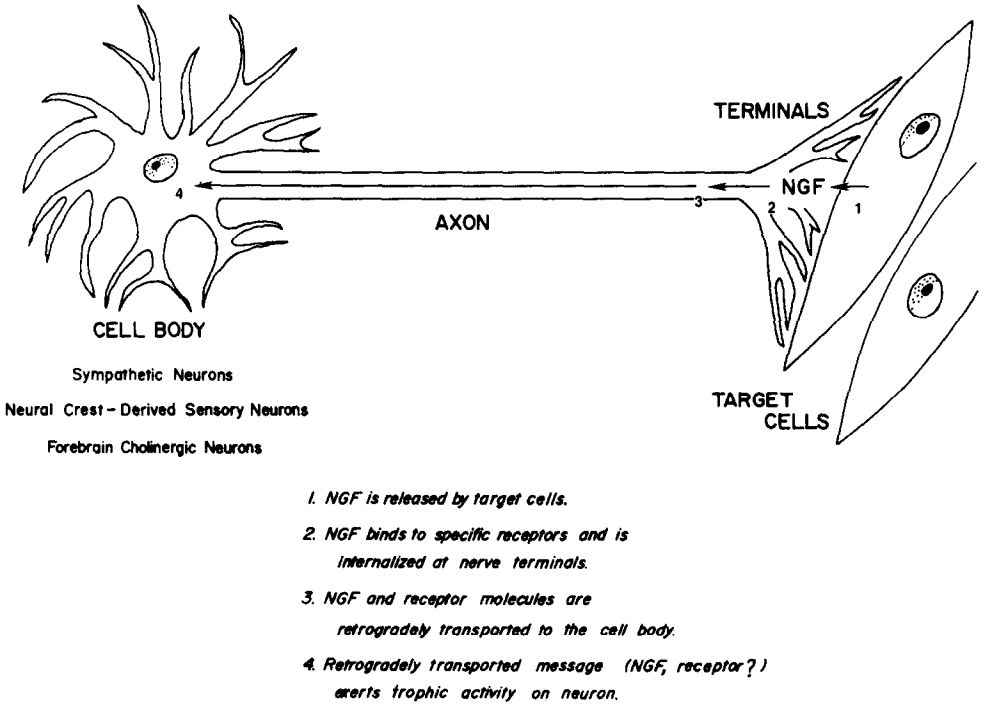

Fig. 1. Diagram showing general scheme by which NGF acts as a target-derived neurotrophic factor on responsive neurons.

sidered to be a target-derived trophic factor acting on certain peripheral neurons. Several recent observations, however, have provided compelling evidence that NGF produces pharmacological, and probably physiological, effects in the central nervous system (CNS). Our charge in preparing this commentary is to discuss this recent literature with particular attention to the NGF receptor in the CNS. This discussion is made timely and of more general interest by other recent developments. In particular, these advances include: (1) the demonstration that a major population of NGF-responsive cell types in the CNS, the forebrain cholinergic neurons, is involved in memory functions and is consistently depleted in patients with Alzheimer's disease; and (2) the development of methods and tools to study the level and distribution of both NGF and the NGF receptor. Our purpose is to summarize briefly the existing literature and to discuss major unresolved questions. It is our view that the effects of NGF on central cholinergic neurons are unlikely to be the only important action of NGF in the CNS. We hope our discussion will provide a useful background that will help place into perspective the burgeoning literature which will come forth over the next few years on this topic.

The NGF receptor

The protein species that constitute the functional NGF "receptor" have not been fully defined. A systematic review of the complex, and sometimes contradictory, literature is beyond the scope of this paper; therefore, only a brief summary is provided. The binding of NGF to NGF-responsive cell types is characterized by nonlinear Scatchard plots which can be explained by the existence of two binding entities [9]. Binding to high affinity sites (K_d 10^{-10}–10^{-11} M) appears to correlate with the biological effects of NGF and with the ability of cells to internalize NGF. Low affinity NGF-binding sites ($K_d \approx 10^{-9}$ M) also exist on most cell types bearing the high affinity sites. These low affinity receptors appear to be incapable of internalizing NGF.

Some cell types, particularly melanomas, Schwann cells, and Schwannomas, possess only low affinity receptors [10–12]. There is very little evidence that NGF is capable of producing effects in these cells. The primary kinetic difference between these two populations of binding sites is in their dissociation rates. The association rates of both populations are rapid and apparently diffusion limited. Their dissociation rates, however, differ considerably. The $T_{\frac{1}{2}}$ of the low affinity sites at 4° is less than 30 sec, whereas the dissociation rate of the high affinity is extremely slow at 4° (little or no dissociation at 0° over a few hours). Because of these differences, the high affinity binding sites have been termed the "slow" receptors and the low affinity species the "fast" receptors. Again, the biological actions of NGF on neurons, or responsive tumor lines such as PC12 cells, are associated with the slow receptors (for review, see Ref. 13). However, there are data suggesting that the binding of NGF can convert the low affinity species to the high affinity species [14]. Therefore, it is possible that the dichotomous binding activities represent not simply two different receptor proteins, but the binding of NGF to a single binding moiety capable of two dissociation states.

The structure of the receptor(s) mediating these

kinetically distinct binding sites is, as yet, not fully understood. Thus, the level of understanding of the NGF receptors is not as advanced as is the case of other factors such as insulin or epidermal growth factor. The reported molecular weights of the binding entities have varied depending on experimental methodologies (hydrodynamic studies, various cross-linking agents ± immunoprecipation). The complex literature has been summarized recently in several papers (e.g. Refs. 15 and 16). Briefly, species with approximate molecular weights ranging from ≈70 kD to 210 kD have been observed. Particularly compelling evidence has been presented, by using cross-linking methods, that the high affinity, slow receptor is associated with a species of $M_r \approx 140$ kD, whereas the low affinity, fast receptor is associated with an M_r of ≈80 kD. Several possible explanations to account for these observations have been put forward, including that there are two distinctly different binding proteins.

Several studies have been reported using monoclonal antibodies that recognize the rat [17] or human NGF receptors [18]. Both of these monoclonal antibodies have been used to immunoprecipitate receptor cross-linked to ^{125}I-NGF with carbodiimide reagents [18, 19]. In both rat and human cells, the major species observed is a 70–80 kD phosphorylated glycoprotein. Using these monoclonal antibodies as selection tools, cDNAs of the human and rat messenger RNAs have been isolated and sequenced [20, 21]. The proteins are highly conserved between the species. In the published results of the human receptor, the molecule is a 427-amino acid, transmembrane protein with a 155-amino acid cytoplasmic domain. Although the receptor is highly conserved in evolution (probes against the human receptor apparently recognize quail receptor mRNA [20]), the amino acid sequence does not bear homology to other known proteins, including receptors for other hormones or hormone-like agents.

Despite the significant advance that the determination of cDNA and amino acid structure provides, it has not resolved the discrepancies in the observed molecular weights of NGF receptor species, nor has it explained the existence of the two kinetically distinct forms. Transfection experiments by the groups who have cloned the human and rat cDNAs have, as yet, produced only cells bearing the low affinity form of the receptor. A model favoured by ourselves and other workers proposes that the 70–80 kD receptor species can, by itself, mediate only the fast dissociating form of the receptor. However, in association with an ≈50–60 kD membrane or cytoskeletal protein, the fast dissociating receptor is converted to the slow dissociating form. This model also suggests that this putative 50–60 kD modifier protein is critical both in transducing the NGF biological signals and in producing internalization of the NGF receptor complex. Work over the next few years will, we hope, provide information on the validity of this or alternative hypotheses, since an understanding of these issues will be a critical step in elucidating the mechanism(s) of action of NGF. A critical point for the present discussion is whether the NGF receptor in the CNS is the same species as that which has been characterized in peripheral cell types. This appears to be the case (see below).

Effects of NGF in the CNS: NGF and basal forebrain cholinergic neurons

Initial experiments searching for NGF effects on CNS neurons focused on the potential ability, based on analogy with peripheral sympathetic neurons, of NGF to affect CNS catecholaminergic neurons. NGF was found to produce no effect on such cells *in vitro* [22] nor was there retrograde axonal transport of ^{125}I-NGF from projection sites to cell bodies (e.g. cortex to locus coeruleus) as occurs in peripheral sympathetic neurons [23]. A critical observation was made, however, during the course of the CNS retrograde transport experiments. Schwab *et al.* [23] observed apparently specific transport of ^{125}I-NGF from areas such as cortex and hippocampus to large cell bodies in the medial septum and the nucleus basalis. The locations of these cells were compatible with those of the basal forebrain cholinergic system. This tentative conclusion was supported by a subsequent, more detailed, examination of the retrograde transport of ^{125}I-NGF in the rat CNS [24]. The central forebrain cholinergic system is composed of large cholinergic neurons whose cell bodies lie in the medial septal nucleus, the diagonal band of Broca, and in the nucleus basalis magnocellularis (nucleus basalis of Meynert). Their axons innervate the amygdala, hippocampus, and neocortex. This rather contiguous group of cells has been implicated in memory functions. Substantial interest and impetus for study of these cells have been provided by the observation that loss of these cells is a consistent neuropathological finding in Alzheimer's disease. Because of the effects (see below) of NGF on these cells, there has been considerable speculation about possible roles of NGF in the pathogenesis or in the treatment of Alzheimer's disease [25].

During the past 3–4 years, other studies have focused attention on the basal forebrain cholinergic system. First, methods were developed to quantitate NGF protein and NGF mRNA levels; these methods were applied to the CNS. The levels of both NGF protein and mRNA generally correlate with the anatomical distribution of basal forebrain cholinergic neurons in rat brain [9, 26–28]. NGF levels are highest in nucleus basalis and lower, but still detectable, in areas not containing projections of basal forebrain cholinergic neurons, such as optic tectum, medulla, and cerebellum. NGF mRNA levels are present in the highest levels in hippocampus and cortex. Developmentally, mRNA and NGF protein markedly increase postnatally in target areas of the cholinergic neurons; NGF levels in basal forebrain rise just prior to increases in choline acetyltransferase (CAT) in the cells [29]. In contrast to the cortex and hippocampus, NGF and NGF mRNA levels change little during postnatal life in the cerebellum. Therefore, it is clear that one criterion for a physiological role of NGF is satisfied in forebrain cholinergic neurons. NGF exists in the central nervous system, and its levels are differentially regulated during development.

Other studies have shown that NGF is capable

of exerting effects on forebrain cholinergic neurons similar to those exerted by NGF on well-documented NGF targets (peripheral sympathetic neurons and neural crest-derived sensory neurons). In tissue culture, the initial observation of Honeggar and Lenoir [30] showed that NGF increases CAT activity in dissociated neurons obtained from the basal forebrain area of embryonic rat. This observation has been reproduced and extended by others. *In vivo* administration of NGF directly into the CNS of newborn rats produces an elevation in CAT activity in areas of both cell bodies and terminal projections of basal forebrain cholinergic neurons [31, 32]. A particularly important experiment from the pharmacological perspective is the recent demonstration that intraventricular administration of NGF can prevent the cell death of medial septal cholinergic neurons caused by axotomy of those cells in lesions of the fornix/fimbria [33–35].

A critical piece of data missing in the case for NGF as a physiologically relevant trophic factor in basal forebrain cholinergic neurons is the lack of demonstrated effects of NGF deprivation on these cells. It must be remembered that effects of an exogenous factor or hormone on any system demonstrate *pharmacological* effects but can only be suggestive of *physiological* roles for the agent. The most compelling evidence for a physiological role is to show an effect of deprivation of the factor. In the case of NGF action in the PNS, this has been accomplished by administration of antibodies to NGF (anti-NGF). Published studies to date have failed to demonstrate an adverse effect (e.g. cell death, atrophy, decreased CAT activity) of anti-NGF on forebrain cholinergic neurons [31]. Obviously, such negative results must be interpreted with caution since several factors (e.g. limited distribution of antibodies *in vivo*, timing, elaboration of other supportive factors *in vitro*) could produce negative results despite a physiological requirement of these cells for NGF, at least during some stage of development. Therefore, despite the evidence described in the above discussion, at the present time it is not yet established that NGF has a physiological function in forebrain cholinergic neurons analogous to that established in peripheral sympathetic and neural crest-derived sensory neurons.

The clearest case for a function of NGF in the CNS has been made for the basal forebrain cholinergic neurons. Evidence for a role of NGF in the CNS on other neuronal types, however, has been presented. The appearance of NGF receptor on the central process of peripheral sensory neurons [36, 37] suggests a possible role for CNS-derived NGF in the development and/or maintenance of sensory neurons. Despite the observations that the distribution of NGF and NGF mRNA generally correlate with the areas of cell bodies or targets of the forebrain cholinergic neurons, both NGF and NGF mRNA are found widely throughout the CNS. Effects of NGF have been shown on striatal cholinergic neurons both *in vivo* [38] and in culture [39]. Further evidence for a broader function of NGF in the CNS comes from very recent studies that have examined the quantities and anatomical distribution of NGF receptors in the CNS.

NGF receptors in the central nervous system

Demonstrations of the specific binding of ^{125}I-NGF to membranes prepared from chick embryo brains [40] and the observation of retrograde transport of ^{125}I-NGF in forebrain cholinergic neurons [23, 24] suggest the presence of NGF receptors on CNS neurons. On the other hand, the failure of anti-NGF to alter the biochemistry or morphology of these or other CNS neurons raises the possibility that exogenous NGF administered to the brain behaves as an agonist for a different, brain-endogenous agent by binding the receptor of the agent—analogously, perhaps, to the ability of insulin to act on IGF receptors. The hypothesis that NGF acts physiologically in the brain has been strengthened by recent evidence showing that specific NGF receptors, apparently identical to those in peripheral tissue, exist widely in brain and, in particular, are found on forebrain cholinergic neurons.

Several approaches have been used to localize and/or quantitate NGF binding sites in the CNS. The first are retrograde transport experiments in which ^{125}I-NGF is injected locally into a target area, Time is allowed for the ^{125}I-NGF to be internalized and retrogradely transported to the cell bodies, and the amount of NGF retrogradely transported is quantitated by determining radioactivity and/or localized by autoradiography. Such studies have shown [23, 24], as mentioned previously, that ^{125}I-NGF is transported from cortex and hippocampus to nucleus basalis and medical septum/diagonal band respectively. Also, a similar paradigm has been used to show that NGF binding sites exist on the central processes of neural crest-derived sensory neurons which can mediate transport of ^{125}I-NGF from the central nervous system to the peripheral nervous system [36, 37]. Although the physiological relevance of the latter observation is not clear, it serves to emphasize an important point; a significant amount of the NGF receptors in areas of CNS (spinal cord, brain stem, midbrain) which receive central projections from neural crest-derived sensory neurons may be produced by cell bodies lying outside the CNS and transported into the CNS.

A second approach has been to quantitate NGF receptors in brain areas. This has been done by using traditional ligand binding assays on membrane preparations but the low levels of receptors in the CNS seriously challenge the sensitivity of such assays. We have developed recently [19] an alternate analytical method to quantitate NGF receptors in CNS which we estimate is approximately fifty times as sensitive as ligand binding assays. The method utilizes a monoclonal antibody (192-IgG; Ref. 17) which binds specifically and exclusively to the rat NGF receptor [19]. The procedure involves incubation of ^{125}I-NGF with intact cells or membranes, cross-linking of the ^{125}I-NGF to the receptor, and subsequent immunoprecipitation of the ^{125}I-NGF:NGF receptor complex. This assay yields increased sensitivity since it requires two specific interactions, similar to two-site immunoassays. ^{125}I-NGF must bind to the ligand occupancy site of the receptor, and the monoclonal antibody must bind to a separate site on the receptor. 192-IgG is also very useful as an alternative to NGF in retrograde trans-

port studies since ^{125}I-192-IgG is retrogradely transported in sympathetic and sensory neurons in a manner identical to ^{125}I-NGF [41]. In addition, monoclonal antibodies can be used as immunohistochemical reagents to localize NGF receptors. A major advantage of the cross-link/immunoprecipitation assay, in addition to sensitivity, is the ability to perform sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and autoradiography and thereby visualize the NGF-bound species in different tissues. By using 192-IgG, we have shown that, in grossly dissected brain areas from adult rats, NGF receptor is widely and rather uniformly distributed, with areas of highest density (medial septum) being only 2.5 times higher than areas such as thalamus [19]. Examination of the apparent molecular weight of receptors in brain areas show a band identical to that seen in sympathetic neurons. Thus, by the criteria of apparent molecular weight and binding of a specific monoclonal antibody, the NGF receptor in CNS is the same as in the PNS. The possibility that the receptor quantitated in brain areas resides on sympathetic innervation of blood vessels was excluded by showing that sympathectomy failed to eliminate receptor in any brain area. In addition, we showed that forebrain cholinergic neurons retrogradely transport ^{125}I-192-IgG in a manner and in an anatomical pattern similar to that previously reported for NGF. Therefore, there seems little doubt that the effects of NGF on basal forebrain cholinergic neurons are mediated by NGF receptors, and there is no need to invoke the idea that the pharmacological effects of NGF so far demonstrated represent NGF acting as an agonist at the receptor for some other endogenous brain ligand.

Somewhat unexpectedly, our studies of the quantitative distribution of NGF receptor in adult rat brain demonstrated high levels of NGF receptor in areas not directly related to forebrain cholinergic neurons: thalamus and cerebellum. Cerebellum, in particular, has levels of receptor as high as medial septum. This very reproducible observation is particularly surprising since levels of NGF protein and NGF mRNA are low in cerebellum [26]. This observation serves to emphasize the point made below that we are likely to find other structures, in addition to cholinergic neurons, on which NGF exerts effects.

A third approach used to study NGF receptors in the CNS has been to localize NGF receptors anatomically by either autoradiography in which ^{125}I-NGF is bound to tissue sections or immunohistochemistry with monoclonal antibodies against primate or rat NGF receptors. The former method has been used in both chicken embryos [42] and in adult rats [43]. The study in chicken embryo did not discuss the distribution of NGF binding sites in brain. However, that study [42] did make an unexpected observation of transient binding of ^{125}I-NGF to ventral spinal cord which appears to correlate with the pattern of motor neurons. The binding was seen between embryonic day 6 (E6) and E8, was reduced by E10, and was gone by E12. Work done in our laboratory (Q. Yan, unpublished)* showed similar transient staining in embryonic rat spinal cord with the monoclonal antibody against the NGF receptor. By using autoradiography in adult rat brain, Richardson *et al.* [43] observed labeled cell bodies, as expected, in basal forebrain cholinergic neurons, but also in other brain areas such as brain stem (e.g. in the cochlear nucleus and dorsal nucleus of the lateral lemniscus). In addition to the labeling of cell bodies in the CNS, central projections from peripheral sensory ganglia could be clearly discerned.

Many investigators are currently studying the distribution of NGF receptors by immunohistochemistry with the monoclonal antibodies against human and rat NGF receptors. The monoclonal antibodies against the human receptor crossreact with receptor in higher primates, but not with receptors in common laboratory species. The antibody against the rat receptor does not recognize other common laboratory species [41]. These monoclonals have proven useful for a variety of purposes (see above) in addition to immunohistochemistry. At the time of this writing the work using the monoclonal antibodies to visualize NGF receptor in CNS remains unpublished except for a paper by Hefti *et al.* [44] clearly showing the staining of human forebrain cholinergic neurons by anti-receptor antibody. Several workers, using both *in vivo* and *in vitro* approaches, have concluded that markers of cholinergic neurons (e.g. choline acetyltransferase immunohistochemistry) colocalize with NGF receptor immunoreactivity in the basal forebrain, i.e. all cholinergic medial septal neurons bear NGF receptors (unpublished). As mentioned above, several workers are currently using immunohistochemistry to localize NGF receptors in developing and mature brain. Some initial reports were presented at the recent Neuroscience Meeting (1986, Washington, DC). Consistent with the autoradiographic data described above, NGF receptor immunoreactivity was reported in structures besides the basal forebrain cholinergic system and was seen transiently in some areas of developing CNS. The latter is consistent with the observation that the density of NGF receptor, measured by the cross-link/immunoprecipitation method decreases several-fold from birth to adulthood [45]. For example, Loy and Koh (unpublished results*) reported receptor immunoreactivity throughout CNS sensory pathways. In preliminary experiments in our laboratory (Q. Yan, unpublished*), staining is associated with cerebellar purkinje and granule cell layer transiently during postnatal development. The physiological significance of these receptors and indeed the cells bearing the observed receptors (neurons, glial elements) are, at this early point, still matters of conjecture.

Concluding comments

The general view of NGF as a peripheral target-derived trophic factor for sympathetic and sensory neurons has been expanded considerably in recent years. NGF within the central nervous system may interact with NGF receptors on the central processes of peripheral sensory neurons [36, 37] and exert trophic effects on these sensory neurons. Of more immediate interest is the clear potential of NGF, made in the CNS, to act on CNS neurons to exert

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effects similar to those seen in the peripheral nervous system. Data that NGF acts physiologically on central forebrain cholinergic neurons in a manner comparable to peripheral neurons (Fig. 1) are compelling, but not yet conclusive. Evidence of an adverse effect of NGF deprivation on these cells is still the missing link in the story. The pharmacological effects of NGF on these cells [30, 31], however, appear to mimic those seen in peripheral neurons, including the ability of NGF to prevent the death of these neurons when they are isolated from their targets by axotomy [33–35].

It would be premature at this point, however, to focus excessively on the forebrain cholinergic system in the examination of NGF in the central nervous system. The widespread, though non-uniform, distribution of NGF mRNA [4, 26–29] clearly places NGF throughout the CNS. The observation of NGF effects on striatal neurons [38, 39] and the widespread distribution of NGF receptor clearly suggest a role for NGF on other neuronal types. The transient appearance of NGF receptors in unexpected places (e.g. ventral spinal cord) during development suggests that the role of NGF may change in many cell types.

It is perhaps even too narrow to view the potential roles of NGF in the context of the target derived trophic factor model (Fig. 1). We and others have observed high densities of NGF receptors on Schwann cells in peripheral nerve during development [12, 18, 45–47,*] and after axonal injury in adult animals [48]. We proposed that these receptors may mediate the sequestration of NGF within developing or regenerating nerve and thereby provide support to growing axons. These recent results in the peripheral nervous system lead to the speculation that not all NGF receptors in the CNS reside on neuronal elements, but rather that some may reside on glial elements and play a role in maintaining or directing growing CNS axons. Progress in understanding the functions of NGF in the CNS will be greatly enhanced by the development of methods and reagents which have become available within the last few years. These include reliable two-site immunoassays for NGF and NGF receptor, probes for both NGF and NGF mRNA which allow quantitation and localization of the mRNAs, and the development of antibodies to the receptor which will help identify neurons potentially able to respond to NGF. These same tools will clarify the roles of NGF and receptors which may exist on non-neuronal elements.

In addition to studies of these developmental and physiological issues, efforts will be made to determine the pharmacological potential of NGF in preventing or ameliorating the effects of damage to central neurons, particularly basal forebrain cholinergic neurons. The possible relevance of NGF in the loss of the forebrain cholinergic neurons in Alzheimer's disease will be explored. Thus, the recent discoveries and appreciation of the actions of NGF in the CNS are likely just the beginning of what will be an active and fruitful area of research for several years to come.

* P. S. Di Stefano and E. M. Johnson, Jr., *J. Neurosci.* in press.

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