The field of neurotrophin biology has made great advances in recent years to include a greater understanding of signaling pathways and broader understanding of the diverse biological roles of these molecules. This review will focus primarily on the nerve growth factor family of neurotrophins and how recent descriptions of the molecular function of both the ligands and the receptors have helped us to understand the basis for many neurologic processes. Ultimately, the goals of such studies are to give us further insight into potential diagnostic and therapeutic uses for these factors or signaling intermediates that may activate given pathways in neurotrophin signaling to achieve a particular objective based upon the underlying disease.

The prototypical neurotrophin is nerve growth factor (NGF), first described by Levi-Montalcini almost 50 years ago. The catalyst for her experiments was the observation that, in mouse sarcomas transplanted into chick embryos, certain tumors secreted a soluble factor that led to large outgrowths of sympathetic and sensory ganglia. In the process of characterizing the roles of NGF, it was discovered that there are essentially 2 functions of this secreted peptide. The first is to support already differentiated neurons in a trophic manner. The second is that NGF is also found to be synthesized by nonneuronal cells distant from the neuronal cell bodies and is responsible for targeting innervation by sympathetic and sensory ganglia at nonneuronal sites, ie, target organs.

After the second neurotrophic factor was isolated and designated brain-derived neurotrophic factor (BDNF), more credence was given to the unproven hypothesis that most neurons respond to and are regulated by neurotrophic factors. Molecular cloning of BDNF revealed it to be structurally related to NGF and using a homology cloning approach, 2 additional members have been cloned in humans and are designated neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5).

**NEUROTROPHIN RECEPTORS**

Insight into the mechanism of neurotrophin action was not significantly appreciated until the cloning and characterization of the neurotrophin receptors. The first receptor identified to be linked to NGF was p75NGFR. This receptor binds neurotrophins at low affinity and, moreover, binds all neurotrophins, thus not demonstrating specificity. The high-affinity neurotrophin-specific receptors are known as trk receptors. These trk receptors were first isolated as the product of an oncogene in colon carcinoma, although expression studies showed that developmentally it was not expressed in colon at all, but rather most strongly in neural crest–derived sensory ganglia. Subsequently, it was identified as the primary signal transducer for NGF and was found to be a tyrosine kinase receptor similar to other previously described tyrosine kinase receptors such as the insulin receptor and the fibroblast growth factor receptor. Other related members of the trk family of receptors were subsequently identified and found to be approximately 85% homologous at the sequence level to the original trk. Three members of the trk family have now been characterized with NGF binding trk A, BDNF and NT-4/5 binding trk B, and NT-3 binding trk C. In addition, there is some lesser affinity binding between ligands and
receptors, as NT-3 also binds both trk A and trk B, and NT-4/5 binds trk C.  

NEUROTROPHIN PHYSIOLOGY

One of the most powerful tools of molecular biology in recent years has been the advent of targeted gene deletions in mice. Such "knockout" mice for each of the neurotrophins and their receptors have shed considerable light on their physiologic functions. Since most of these mice that have homozygous deletions of either neurotrophin or receptor are not viable beyond the early neonatal period, particularly close attention has been paid to perturbations in development. Both NGF and trk A knockouts have resulted in at least partially predictable phenotypes. The animals are viable at birth but smaller than their wild-type littermates. Both knockouts have dramatically reduced dorsal root ganglia and trigeminal neurons at birth, and within 10 days, the sympathetic ganglia have nearly disappeared. In the central nervous system, the phenotype is less predictable. Early studies of NGF showed that basal forebrain cholinergic neurons express trk A and are responsive to NGF. In the knockout animals, these neurons survive as long as the animals do, suggesting that neurons in the central nervous system may not be dependent on NGF for differentiation and early survival, though it may be critical for proper functioning.

Brain-derived neurotrophic factor and trk B knockouts also exhibit striking phenotypes. Trk B knockouts in which both BDNF and NT-4/5 function is abolished die within 48 hours due to a variety of neural deficits. For example, neuronal losses are found in sensory ganglia including dorsal root ganglia and trigeminal neurons. Motor neurons also show some losses, though not as severe as sensory populations. Grossly, the central nervous system appears normal morphologically, but more recent data have shown that there are both morphologic and electrophysiologic deficits, which may reflect roles for BDNF independent of neuronal survival, such as synaptogenesis and synthesis of neurotransmitters. Likewise, NT-3 and trk C knockouts have similarly shown dependence on neurotrophins in certain populations of sensory ganglia. In contrast, NT-4/5 mutant mice are morphologically normal and can reproduce, though they do have some deficits in sensory ganglia.  

NEUROTROPHIN SIGNALING

The knockout models have been extremely useful in delineating neurotrophin function during development, but their roles in fully differentiated neurons are still largely unknown. The mediators of neurotrophin signaling have now been studied and give insight into how neurotrophins may instruct neurons to do different things based on a given cellular environment. PC-12 cells have been used as a model system for studying neurotrophin signaling because they express trk A and, when exposed to serum or NGF, differentiate into neuronal-like cells and extend axonal processes. Trk A signaling has been shown to occur via at least 2 distinct pathways. Phosphorylated trk A associates with docking proteins Shc and Grb2, which then activate either the Ras or phosphatidylinositol-3 (PI3)-kinase pathways. The Ras pathway activates a series of kinases that includes Raf, mitogen-activated protein kinase--Erk kinase (MEK), and ultimately mitogen-activated protein kinase (MAPK), which translocates to the nucleus where it phosphorylates transcription factors. The PI3-kinase pathway can be activated by trk A directly through Shc and Grb2 or alternatively through activated Ras. Recently, these pathways have been further distinguished using recombinant adenoviruses to express intermediates of these pathways in constitutively active and dominant negative forms, which implicate the Ras pathway for neuronal differentiation and the PI3-kinase pathway for neuronal survival. Thus, depending on which pathway dominates, cells can be induced to grow and differentiate or just get enough trophic support to survive. These 2 distinct signaling pathways have direct implications for in vivo delivery of neurotrophins as to what the therapeutic objective may be.  

A third signaling pathway similarly carries clinical implications as to how the presence of neurotrophins may alter cell fate. The p75NGFR, or low-affinity pan-neurotrophin receptor, belongs to the tumor necrosis factor receptor superfamily and also appears to mediate distinct cellular processes. In the presence of trk A receptors, p75NGFR participates in the formation of high-affinity binding sites and enhances neurotrophin responsiveness, which can lead to increased neuronal survival. In the absence of trk A, however, p75NGFR in certain cell populations can activate a cell death signal. The signaling mechanisms underlying these opposed responses have not been fully worked out, but activation of c-Jun N-terminal kinase (JNK) has been observed in cells undergoing cell death as well as activation of nuclear factor kappa B (NFkB). Both JNK and NFkB are modulated by tumor necrosis factor receptor--associated factors (TRAFs), and recently TRAF6 has been shown to associate with p75NGFR.

Thus, depending on which signaling pathway is activated, neurotrophins may lead to cell survival, growth and differentiation, or cell death. Now that there is some rudimentary understanding of how signaling occurs, it is more apparent why neurotrophin therapy in neurologic conditions has thus far had limited success. In applying neurotrophic therapy to neurologic disease, there needs to be a systematic approach to tailor therapy to particular conditions based on the underlying disease. Examples of neurologic diseases for which neurotrophin therapy has been most extensively studied include diabetic neuropathy and amyotrophic lateral sclerosis in the peripheral nervous system, and Alzheimer and Parkinson disease in the central nervous system.  

RELEVANCE OF NEUROTROPHINS TO NEUROLOGIC DISEASE

The potential for neurotrophin therapy in the peripheral nervous system is obvious since most of the
characterization of neurotrophin function has been with peripheral sensory ganglia. Most recent efforts have been aimed at treating diabetic polyneuropathy and amyotrophic lateral sclerosis. Diabetic neuropathy is characterized by the degeneration of large- and small-diameter sensory fibers, which mediate pain and temperature, and large-diameter myelinated sensory fibers that convey tactile sensation and proprioception. Studies have shown that decreased availability to NGF contributes to its pathogenesis, and retrograde axonal transport of NGF is impaired in diabetic models. It is this retrograde transport that enables NGF-dependent neurons to survive. These data have led to clinical trials of subcutaneous injections of NGF, and a phase 2 study has shown promising results, with a large 1500-patient phase 3 trial currently under way.12

The 2 most common motor neuron diseases are spinal muscular atrophy and amyotrophic lateral sclerosis. These diseases are characterized by degeneration of lower and in some cases upper motor neurons. There is a wealth of data showing that neurotrophins including BDNF and NT-3 as well as other factors such as ciliary neurotrophic factor, glial-derived neurotrophic factor, and members of the fibroblast growth factor family all support in vitro motor neuron survival. Thus far, clinical trials with these agents have been disappointing, related to side effects and poor delivery to neurons as the growth factors have fairly short half-lives. One approach to circumvent this delivery problem, which has now shown to be promising in a murine model of motor neuron atrophy, is a gene transfer method of delivering the gene itself via adenoviral vectors.13

Much of the effort at understanding neurotrophin roles in the central nervous system has focused on the basal cholinergic forebrain and the dopaminergic system in the substantia nigra. These areas show profound degeneration in Alzheimer and Parkinson diseases, respectively. Nerve growth factor is known to support growth for cholinergic neurons in the basal forebrain. The results of anecdotal reports of NGF infusions in the brains of patients with severe Alzheimer disease-associated dementia have been equivocal. More recently, efforts to supply the forebrain with a stable supply of NGF via transplantation of an NGF-secreting cell line have shown more promise in animal models of dementia.14 In Parkinson disease the rationale for infusing the basal ganglia with NGF has been centered around the well-described model of PC-12 cells being induced to differentiate and survive into neuronal-like cells when exposed to NGF. PC-12 cells are a commonly used, immortalized cell line derived from rat pheochromocytoma. Thus, adrenal implantations into the basal ganglia of patients with Parkinson disease have transient effects, but when cotransplanted with NGF, the chromaffin transplants are hypothesized to transform into differentiated neurons and then endogenous NGF secretion can provide trophic support of these neurons.15

THE FATE OF NEUROTROPHIN THERAPY FOR NEUROLOGIC DISEASE

Thus far, clinical trials using neurotrophins in neurologic diseases based in both the peripheral and central nervous systems have been disappointing. Even the most promising studies, such as NGF treatment of peripheral neuropathies, are associated with unwanted side effects related to drug delivery. It appears that rather than overwhelming a given anatomical area with an abundance of neurotrophin substrate, a more rational approach would be to direct therapy toward particular diseased neurons in a way that will obtain a therapeutic objective while minimizing unwanted side effects. There are 2 areas in which this is being pursued. The first is by tailoring synthetic neurotrophin-like molecules, and the second centers around our understanding of the mediators for receptor signaling.

The crystal structure of NGF is now known and there are many models of neurotrophin–trk interactions that have been proposed. Several different approaches have identified immunoglobulin-like domains of trk A as those critical for NGF binding.10 Likewise, the critical amino acids required for neurotrophin–trk binding have been identified and are believed to convey
specificity of trk activation. They have also contributed to our understanding of how neurotrophins convey their signals. Expression of neurotrophins and the trk receptors is anatomically more widespread and found in more cell types than many other ligand-receptor systems. Thus, identifying the appropriate binding domains of neurotrophins and their cognate receptors is essential if a therapeutic objective is to only signal a limited population of neurons. As more is understood about such binding, it will become easier to develop synthetic peptides that bind to all cells expressing trk receptors, but only affect signaling in the select few that are relevant to the impaired neurologic process.

As has been shown in the Figure, neurotrophin signaling, under the right set of circumstances, can prompt a cell to survive, grow and differentiate, or undergo apoptosis. At present, there is limited understanding of which neurons will act in which way when stimulated by a given neurotrophin. As our understanding of the molecular pathways of trk and p75NGFR signaling has advanced, the prospect of using signaling intermediates as therapeutic agents becomes more provocative. An obvious caveat to such a strategy is that many receptor systems use these common signaling intermediates. To customize therapeutic agents for systemic therapy in neurologic disease, novel agents need to be devised that incorporate unique binding to cells responsive to neurotrophins, as well as selective activation of signaling pathways that will enable neurons to survive or differentiate rather than die.

Accepted for publication July 21, 1999.

Corresponding author: Luis F. Parada, PhD, Center for Developmental Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235-9133 (e-mail: parada@utsw.swmed.edu).

REFERENCES