

Part One: Neurotrophic Factors - An Overview

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Many organic molecules, including hormones and proteins that act in autocrine or paracrine modes, dramatically affect the general maintenance and growth of cells. Collectively, these organic substances define a broad class of molecules known as growth factors, which have been studied intensively for decades. More than 40 years ago a protein growth factor was described, opening a new field of investigation that is flourishing today. The factor, now known as nerve growth factor (NGF), has remarkable trophic effects on neurons (1). In Part One of this two part article, we review NGF and related neurotrophic factors - proteins which are able to promote differentiation, mitosis and/or survival of peripheral and CNS neurons. We review the mechanism of action of a subclass of these proteins known as neurotrophins, of which NGF is the original member (2). In part two of this article, to appear in Promega Notes 51, we will focus primarily on the functions of neurotrophins in development and disease.

Introduction

Neurotrophic factors ([Table 1](#)) are endogenous proteins that regulate the development, maintenance and survival of neurons. Recent evidence suggests that neurotrophic factors also may be implicated in the normal functional activity of nerve cells and may play a role in plasticity. These molecules are generally small, soluble proteins with molecular weights between 13 and 24kDa and are often active as homodimers.

Table 1. Examples of Proteins Reported to Have Neurotrophic Properties.

Proteins with well-documented neurotrophic activity	Proteins with putative neurotrophic activity
Acidic fibroblast growth factor (aFGF)	Cholinergic neuronal differentiation factor (CDF)
Basic fibroblast growth factor (bFGF)	Epidermal growth factor (EGF)
Brain-derived neurotrophic factor (BDNF)	Heparin binding neurotrophic factor (HBNF)
Ciliary neurotrophic factor (CNTF)	Insulin
Interleukin 1, 3 and 6 (IL-1,3,6)	Insulin like growth factors (IGFs)
Neurotrophin-3 (NT-3)	Protease nexin I and II
Neurotrophin-4/5 (NT-4/5)	Transforming growth factor alpha (TGFalpha)
Nerve growth factor (NGF)	
Glial-derived neurotrophic factor (GDNF)	

Neurotrophic factors

The neurotrophic factors, a subclass of growth factors, act on neurons as well as other non-neuronal cells. As depicted in [Figure 1](#), this subclass of factors contains at least three distinct families: the neurotrophins (e.g., NGF; brain-derived neurotrophic factor, BDNF; and

neurotrophins NT-3 and NT-4/5); the neurotrophic cytokines (e.g., ciliary neurotrophic factor, CNTF); and the fibroblast growth factors (e.g., acidic and basic FGF). The members of these families of neurotrophic factors demonstrate significant (50% or more) homology and have affinities for particular classes of receptors. Neurotrophins bind to a low affinity receptor (p75) and to a family of closely related high affinity glycoprotein tyrosine receptor kinases (Trk molecules). The receptor topology for the neurotrophic cytokines is very different. The transduction pathway for CNTF, for example, involves a tyrosine kinase, but the receptor itself is not a tyrosine kinase; the CNTF receptor consists of a complex of three subunits, only one of which directly binds CNTF (3, 4), and the tyrosine kinase JAK/TYK, which associates with the CNTF receptor complex on the cytosolic side of the plasma membrane. The binding of the FGFs on the other hand is different from the other neurotrophic factors in that it involves a low affinity binding site related to heparin sulfate and a high affinity receptor that contains a tyrosine kinase (5).

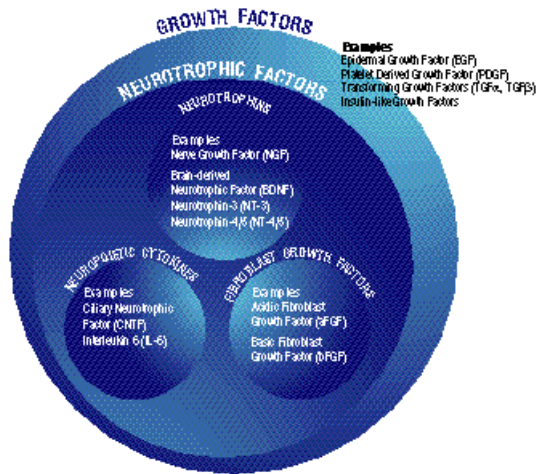


Figure 1. Classification of neurotrophic factors.

Unlike most known receptor molecules, all these tyrosine kinase receptors possess only one transmembrane domain. The first step in their activation by growth factors always involves receptor oligomerization, which seems to be the initial signal transduction event that leads to tyrosine kinase activation. Not only are the receptor mechanisms comparable for the various neurotrophic factors, but also the intracellular signaling pathways are very similar, even for such diverse molecules as NGF, CNTF and FGF (6). Thus, a large group of diverse factors act on distinct receptors, leading to similar intracellular molecular changes which can profoundly impact cell development and survival.

Neurons use numerous routes for incorporating these factors (Figure 2). A neuron may acquire neurotrophic factors from 1) its target tissue, whether neuronal or non-neuronal (target-derived acquisition); 2) neighboring neurons and other cells (paracrine acquisition); or 3) itself (autocrine acquisition). For example, it is well known that during neural development excess neurons are generated. The survival and differentiation of an appropriate number of neurons is largely regulated by target-derived neurotrophic factors. Developing neurons that fail to make connections with their targets will be deprived of necessary neurotrophic factors and will die. Those neurons that establish satisfactory connections survive and go on to differentiate and mature.

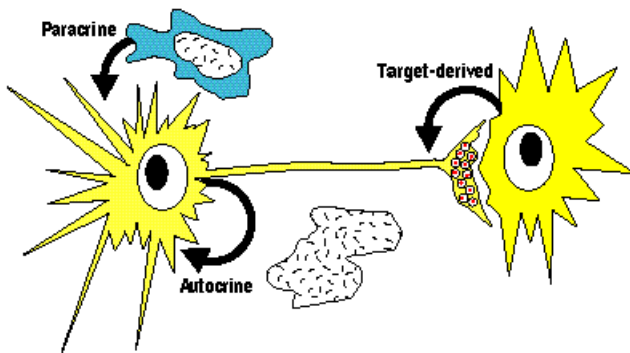


Figure 2. Modes of neurotrophic factor acquisition.

Neurotrophins

The neurotrophins comprise a group of highly homologous proteins that are secreted by cells and act in target-derived, autocrine or paracrine fashion on cell surface receptors. While these growth factors have many effects on neurons, the two most profound effects are promotion of differentiation and survival during development. It is now recognized that both these actions also may be maintained in adult brains, implicating neurotrophins in plasticity and the response to injury in adult and young animals (2).

Neurotrophin receptors

The known receptors for the neurotrophins include three different glycoprotein tyrosine kinases (TrkA, TrkB and TrkC; see [Figure 3](#)) which are similar in nature to the receptors for the non-neuronal growth factors FGF and EGF. The different neurotrophins bind to these receptors with high affinity ($K_{1/2} \sim 10^{-11}$) and with specificity: NGF binds to TrkA, BDNF and NT-4/5 bind to TrkB and NT-3 binds to TrkC. A low affinity receptor, p75, binds NGF and the other neurotrophins with an approximate $K_{1/2}$ of 5×10^{-9} , thus p75 has been termed a pan-neurotrophin receptor (7). The immediate effect of neurotrophins binding to p75 is unknown - there is no apparent associated kinase activity and the functional importance of this binding is unclear. Conflicting data as to the necessity of p75 for neurotrophin action exists for NGF and the other neurotrophins (7,8). p75 knock-out mice demonstrate abnormal development of sensory neurons (8), yet there are several cases where neurotrophins act normally in the absence of p75 (8). For example, it is clear that BDNF produces a normal signaling cascade in cells which lack p75 (9). One potential role for p75 may be to enhance the function of the Trk receptors (7). In addition to p75 and the Trk receptors, truncated TrkB and TrkC receptors, which lack the intracellular tyrosine kinase activities, are widely distributed. It is hypothesized that these proteins are involved in the inactivation of neurotrophins, however, there is no direct evidence for this hypothesis. The neurotrophins have far-reaching effects on cell function and all known cellular effects mediated by these factors result from the tyrosine kinase activities of the receptors. Inhibition of tyrosine kinase activity with the fungal metabolite K252a, a specific inhibitor of the Trk receptors, and mutations affecting tyrosine kinase function block all known biological effects of the neurotrophins (7).

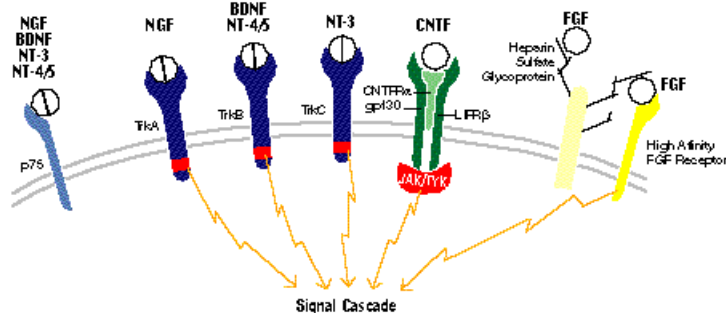


Figure 3. Neurotrophic factors and their receptors. **Neurotrophin receptors** (blue): All neurotrophins bind with low affinity to p75, which has no defined role in neurotrophin signaling. The neurotrophins selectively bind to high affinity Trk receptors and cause the Trk receptor to form homodimers and autophosphorylate, thereby triggering the intracellular signaling cascade. **Neurotrophic cytokine receptors** (green): CNTF binds with low affinity to the specific CNTF alpha-receptor moiety (CNTFR-alpha) which lacks a transmembrane domain. The CNTF:CNTFR-alpha complex interacts with two membrane spanning signaling molecules, gp130 and LIFR-beta, thereby activating an associated tyrosine kinase (JAK/TYK) which transduces the initial intracellular signal. **Fibroblast growth factor receptors** (yellow): FGF binds with low affinity to a heparin sulfate proteoglycan FGF receptor which then facilitates binding of FGF monomers to the high affinity FGF receptor. This interaction leads to receptor autophosphorylation and signal transduction.

Note: Only principal ligand:receptor interactions are shown. High affinity receptors for a specific class of neurotrophic factors are represented with a dark color, while the low affinity receptor has a lighter shade of that color. Tyrosine kinase domains are shown in red.

Intracellular signaling pathways of the neurotrophins

Following the binding of homodimeric neurotrophins to Trk receptors and resultant receptor dimerization, downstream neurotrophin actions occur in two intracellular stages (10). In many ways, these events resemble the actions of other tyrosine kinase-coupled growth factors.

The first cytosolic events are initiated by autophosphorylation of the tyrosine receptor kinase. Autophosphorylation appears necessary for further action of the growth factors - mutant receptors that lack or carry modifications of one or more of these phosphorylation sites lose much, if not all, function (10). Autophosphorylation is thought to expose binding sites on the receptor's cytoplasmic tail for a number of signaling molecules that have sequence homology to a particular binding domain of the *src* proto-oncogene, the SH2 domain (and often to the SH3 domain). These signaling proteins are activated by the binding and, in several cases, by subsequent tyrosine phosphorylations mediated by the receptor kinase activities (8,10). The activation of signaling proteins rapidly leads to downstream activation of several important regulatory proteins, including p21Ras, MAP kinase and ribosomal S6 kinase II (p90). These proteins, in turn, initiate important cytosolic functions, including protein biosynthesis and probably other functions, such as increased membrane

calcium permeability and very probably cytoskeletal rearrangement (7).

The second stage of the signaling process is gene transcription within the nucleus. This is mediated by MAP kinase, S6 kinase II and possibly other activated regulatory proteins which stimulate response elements, thereby inducing transcription of early genes such as *c-fos*, *c-jun* and *c-myc* (11). This stage of the signaling process is generally thought to account for the most profound effects of the neurotrophins, such as neuronal survival and neurite outgrowth. As of now, the relative roles of the cytoplasmic and the nuclear events have, in fact, not been well delineated and this critical area of research is becoming a major focus for many neuroscientists. Most of the above scheme has been deduced from studies on model systems such as PC12 cells and fibroblasts; thus little distinction has made to date between the different receptors and the specific pathways activated by various neurotrophins.

One recent study on hippocampal pyramidal neurons (9) demonstrated autophosphorylation of TrkB (gp145) by BDNF within five minutes as well as activation of MAP kinase within one minute. These cytoplasmic events were followed thirty minutes after treatment by expression of the early gene product c-Fos. Thus, in broad outline, the above scheme is likely to be applicable to the different neurotrophins.

There have been many detailed biochemical studies on various growth factor signaling pathways, including neurotrophins (7,8,10), and recent findings on NGF signaling via TrkA are reviewed in the companion article, [Signal Transduction of Neurotrophin Receptors: Basic Concepts and Available Pharmacological Tools](#), in *Promega Notes Magazine v.50*. Despite the numerous phenomenological studies on the effects of growth factors on development and cell survival (12), the mechanisms by which the biochemical signaling pathways are converted into functional changes remain very poorly defined (10). Interestingly, some clues have been provided by recent studies on the protective effects of these neurotrophins on injured neural tissue. This will be the subject of the second part of this review, which will appear in *Promega Notes 51*.

References

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Ordering Information

Product	Size	Cat. #
NGF, 2.5S, Murine	100µg	G5141
NGF, 7S, Murine	100µg	G5151
rhNT-3	5µg	G1501
rhNT-4	5µg	G1511
rhBDNF	5µg	G1491
rrCNTF	5µg	G1481
rhFGF, Acidic	10µg	G5061
rhFGF, Basic	25µg	G5071
Anti-Human NGF mAb	50µg	G1131
Anti-Human NGF pAb	20µg	G1541
Anti-Human NT-3 pAb	200µg	G1651
Anti-Human NT-4 pAb	200µg	G1621
Anti-Human BDNF pAb	200µg	G1641
Anti-Rat CNTF pAb	200µg	G1631

----- Anti-Bovine FGF, Acidic IgG pAb	1mg	G5081
----- Anti-Bovine FGF, Fragment, Acidic IgG pAb	1mg	G5261
----- Anti-Bovine FGF, Basic mAb	500µg	G5271
----- Anti-FGF Receptor IgG pAb	250µg	G5102

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