

CHAPTER

29

Growth Factors

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INTRODUCTION: WHAT IS A GROWTH FACTOR?

Growth factors are proteins that regulate many aspects of cellular function, including survival, proliferation, migration and differentiation. In non-neuronal cells growth factors stimulate proliferation, but mature neurons are postmitotic and cannot re-enter the cell cycle. Consequently, when considered in the context of the nervous system, growth factors are frequently referred to as neurotrophic factors. These factors are critical for proper development of the nervous system from the earliest embryonic stages. Growth factors determine the fate of cells as they differentiate from being progenitors along either neuronal or glial lineages. In addition, during embryonic development, growth factors are crucial for regulating neuronal survival, determining cell fate and establishing proper connectivity. Many growth factors have now been identified that function in the brain, even factors that were originally identified in other systems, and there is an ever-expanding landscape of growth factor interactions with cellular populations in the nervous system, both during

development and in the adult. The nervous system is composed of an extremely heterogeneous population of cells. In addition to the broad categories of neurons, astrocytes and oligodendrocytes, there are multiple types of neurons with a diversity of structure, function, localization, phenotype and projections, each with specific needs for trophic support. Understanding the complexity of these relationships is a major challenge (see Chaps. 1, 6, 28).

In this chapter the neurotrophin (NGF) family of factors, which were the first growth factors to be identified for actions in the nervous system, will be emphasized. Several other families of growth factors that have important functions in the peripheral and central nervous systems, including the GDNF family, the neuregulins, and the neurotrophic cytokines will be discussed. Finally, other factors that were initially discovered in non-neuronal systems and subsequently shown to have important roles in nervous system function will be touched upon. An important conclusion is that numerous growth factors, whether they were initially discovered in the nervous system or for effects on other cellular populations, have effects on neuronal and glial survival, development and function.

NEUROTROPHINS

The first neural growth factor identified was nerve growth factor (NGF), which was shown by Rita Levi-Montalcini to be essential for survival and neurite outgrowth of peripheral sympathetic neurons and a subpopulation of sensory neurons (Levi-Montalcini & Angeletti, 1963), work for which she was awarded the Nobel Prize in Physiology or Medicine in 1986 together with Stanley Cohen. NGF is produced and secreted by the targets of peripheral neurons, and is present in limiting amounts, leading to competition among the afferent axonal processes for access to this factor. More neurons are generated than are present in the adult, and there is a critical period of cell death during development when the afferent axons compete for access to the trophic factor (see Ch. 28). The neurons that compete successfully for the target-derived trophic factor survive and form stable connections, while those that fail to gain access to the factor undergo cell death (Figure 29-1). Many studies have shown that providing an extra target (by grafting), or an excess of the neurotrophic factor itself, increases the number of surviving afferent neurons, while removing a peripheral target decreases neuron number. This process gave rise to the “neurotrophic factor hypothesis” in which the amount of target determines the number of surviving innervating neurons (Levi-Montalcini & Angeletti, 1968; Oppenheim, 1991). This mechanism suggests that the target secretes the neurotrophic factor; the ligand then binds to receptors at the axonal terminal and is internalized and retrogradely transported to the neuronal cell body. Although this concept was developed based on studies in the PNS, neurons in the CNS also depend on neurotrophic factors that may be derived from their targets. However, targets are not the only source of growth factors. Factors that influence survival and differentiation may be derived from glial cells, including astrocytes, oligodendrocytes and microglia in the CNS, and Schwann cells in the periphery. Glial cells are an important source of growth factors, particularly during development when the growing axons have not yet reached their targets. In peripheral neurons, Schwann cells also provide trophic support for regenerating neurons after injury to facilitate axonal growth and direct them to the denervated target.

In the early 1980s a second neurotrophic factor, brain-derived neurotrophic factor, BDNF (Barde et al., 1982) was purified from brain. The DNA sequence of BDNF showed a high degree of similarity with NGF in specific domains, suggesting that these factors may be members of a gene family (Barde, 1994). The gene family was named “neurotrophins” and the identification of two additional members of this family in mammalian species, neurotrophin-3 (NT3) and neurotrophin-4 (NT4), quickly followed. All the neurotrophins have an approximate molecular weight of 13 kDa and exist as non-covalently linked homodimers. Genetic deletion of each of the neurotrophins in mice has provided information on the role of these factors in supporting survival of different populations of neurons. Frequently, an entire peripheral neuron population is lost when a single neurotrophin is absent, while the CNS of these knockout mice do not show loss of significant populations of neurons. Thus, it has been much more straightforward to assess the trophic requirements of peripheral neurons than CNS neurons, which appear to be supported by

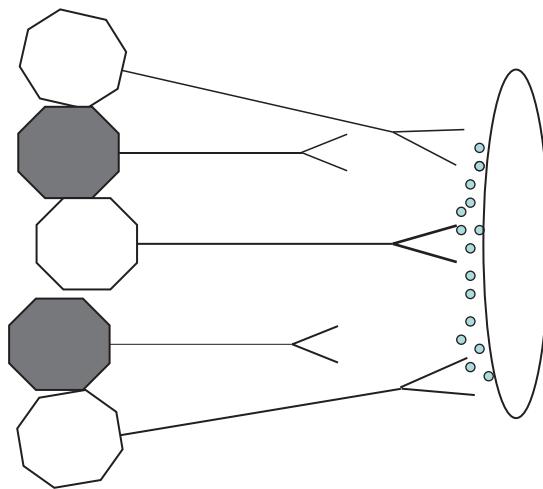


FIGURE 29-1 The “neurotrophic factor hypothesis.” Target tissue synthesizes and releases limiting amounts of neurotrophic factor. Afferent axons compete for access to this factor. Neurons that compete successfully survive and make functional connections (white), while neurons that do not compete successfully, die (gray).

multiple factors that can provide compensatory support for the absence of a single factor. For example, spinal cord motor neurons can be influenced by numerous growth factors, and the removal of one single factor does not result in the loss of the entire neuronal population. These observations also suggest that, although different factors may compensate for the absence of a single factor in supporting neuronal survival, they are not likely to be functionally redundant when all the factors are present. Thus, each neurotrophin may have specific and distinct functional effects on a target population.

In order for any growth factor to have an effect, the responding cells must express the corresponding receptor for that factor. Neurotrophins interact with two cell surface receptors; a member of Trk family of receptor tyrosine kinases and the p75 neurotrophin receptor (p75NTR) (Figure 29-2) (Chao, 2003). See also tyrosine kinases in Chap. 26.

Nerve growth factor

Target-derived NGF is absolutely critical for development and maintenance of peripheral sympathetic and some sensory neurons. The secretion of NGF in limiting amounts by the peripheral targets determines the number of surviving sympathetic neurons in the mature animal. However, neurotrophins do more than regulate neuronal survival. NGF also influences differentiation by regulating extension of axonal and dendritic processes. Furthermore, NGF determines the neurotransmitter phenotype of sympathetic neurons by regulating the expression level of the enzyme tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, thereby promoting the noradrenergic phenotype of these neurons. NGF also supports the survival of a subpopulation of sensory neurons in the dorsal root ganglia (DRG), specifically the small-caliber nociceptive fibers that mediate pain neurotransmission. While these sensory neurons depend upon NGF for their survival during early development, they

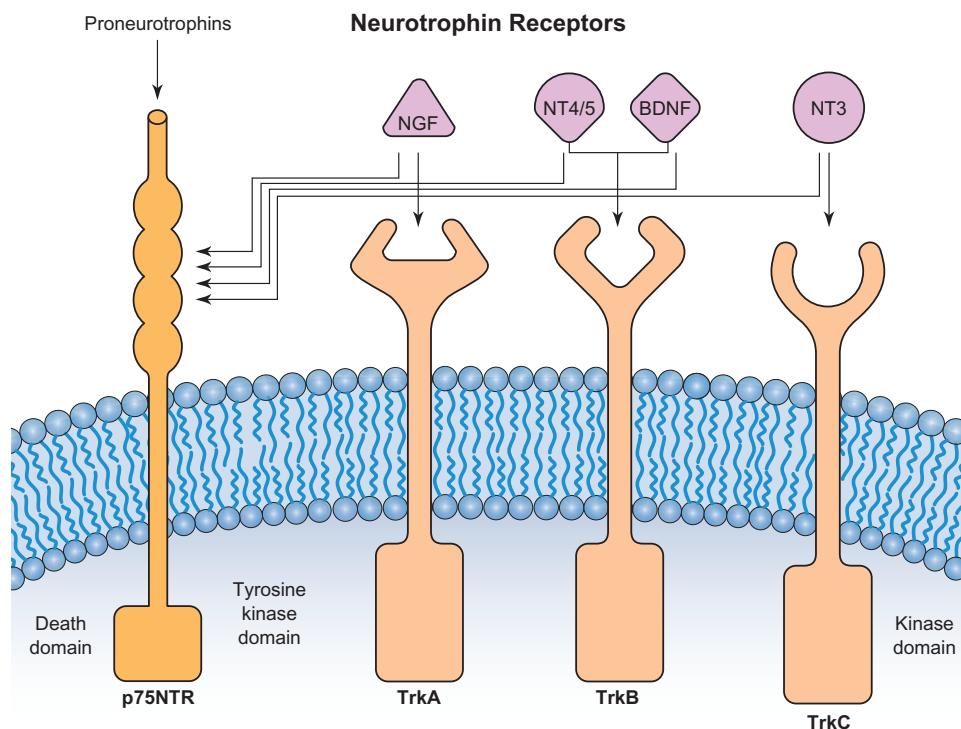


FIGURE 29-2 **Neurotrophin receptors.** Each neurotrophin binds to its cognate Trk receptor. The p75NTR can bind all the neurotrophins with low affinity, and the proneurotrophins with high affinity.

lose their dependence on this factor in the adult. However, NGF continues to affect the function of these neurons by altering the threshold for pain neurotransmission (see Box 29-1). Peripheral inflammation is accompanied by increased levels of NGF, which then contributes to elevated pain sensitivity due to the effects on the nociceptive sensory fibers. In mice with a deletion of the NGF gene, these nociceptive neurons are absent, as are the sympathetic neurons, and these animals cannot survive long after birth.

In the central nervous system NGF is important for maintaining the integrity of afferent cholinergic neurons from the basal forebrain (Fischer et al., 1987). This growth factor is expressed at very low levels, primarily in the cortex and hippocampus consistent with the target-derived mechanism of action defined for peripheral neurons. If these neurons are damaged, they can be rescued from dying by the administration of NGF. The basal forebrain cholinergic neurons are particularly vulnerable to degeneration in Alzheimer's disease, and a potential therapeutic role for NGF has been explored for the treatment of this disease. A major challenge in treating neurodegenerative diseases in the brain is that proteins such as neurotrophins cannot cross the blood-brain barrier. Initial studies providing NGF peripherally did not succeed, as the factor did not reach the affected neurons in the basal forebrain, and moreover caused a side effect of inducing pain, due to the effects of NGF on the nociceptive sensory neurons mentioned above. However, novel strategies for delivering proteins into the brain, including implanting cells that secrete specific factors, have suggested that exogenous NGF provided locally

within the brain can rescue degenerating basal forebrain neurons without the peripheral side effects (Tuszynski et al., 2005).

Brain-derived neurotrophic factor

In the PNS, BDNF influences the survival of specific populations of sensory neurons, including a subpopulation of DRG neurons as well as neurons in the nodose ganglion that do not respond to NGF. Mice lacking BDNF show significant loss of sensory neurons throughout the cranial and spinal sensory ganglia (Ernfors et al., 1994; Jones et al., 1994), as well as a loss of vestibular sensory neurons in the ear. In addition to effects on neuronal survival, BDNF has also been shown to regulate the function of non-neuronal cells. This factor plays an important role in the initiation of myelination by Schwann cells, and in differentiation of oligodendrocytes during development and after injury, influencing the myelinating cells of both the peripheral and central nervous systems in distinct ways. Thus, it is important to realize that these neurotrophic factors affect not only neuronal survival, differentiation and function, but also glial function and interactions between neurons and glia.

BDNF is expressed widely throughout the brain, where it supports survival of specific populations of CNS neurons. These include neurons that are lost in a variety of neurodegenerative diseases, such as spinal cord motor neurons (which degenerate in amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease) and substantia nigra dopaminergic neurons (which degenerate in Parkinson's disease), as well as the basal forebrain cholinergic neurons that degenerate in

NGF AND PAIN

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Chronic pain is a condition suffered by many thousands of individuals and met with inadequate available treatments. The two classes of treatments currently in use, nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid drugs, are not always effective for different types of pain, and can have deleterious side effects. Novel strategies are currently being developed based on the effect of NGF on nociceptive neurons (Hefti et al., 2006; Pezet & McMahon, 2006).

There are many indications that NGF plays a role in pain processing. Increased production of NGF is associated with injury and the levels are maintained in situations of chronic pain and blocking NGF strongly attenuates pain under these conditions. Deficiencies in NGF production are associated with a reduction in pain, and congenital insensitivity to pain has been associated with mutations in the NGF, TrkA and their downstream signaling genes. Focused administration of NGF evokes a robust and prolonged hyperalgesic response in animals. In clinical trials that administered NGF therapeutically to treat peripheral neuropathies, one of the critical side effects was severe hyperalgesia, even in healthy control subjects.

NGF promotes the survival and function of the nociceptive subpopulation of sensory neurons within the DRG during development, and many of these neurons continue to express TrkA and are functionally responsive to NGF throughout life. NGF treatment of sensory neurons elicits release of substance P and CGRP, two neuropeptides that are involved in pain neurotransmission. Additionally, NGF induces the synthesis and release of BDNF from the nociceptive neurons, which acts as a modulator of central nociceptive neurons in the spinal cord. NGF can also modulate the activity of a variety of ion channels involved in nociception, in particular the transient receptor potential vanilloid receptor 1 (TRPV1) channel. NGF increases expression of TRPV1 mRNA, facilitates insertion of these channels in the membrane resulting in a greater density of channels, and can lead to phosphorylation and greater activity of the channel, causing increased sensitivity to capsaicin. Thus, NGF regulates many aspects of nociceptive function in adulthood (Pezet & McMahon, 2006).

A strategy to block NGF either by sequestering the factor itself or blocking its binding to TrkA has proven to be efficacious in ameliorating pain in several animal models. Treatment with anti-NGF agents has diminished or reversed hyperalgesia in chronic inflammatory conditions in which the two established pain treatments, NSAIDs and opiates, either have limited efficacy or adverse side effects.

Strategies for modulating NGF in the treatment of pain

Strategies for reducing NGF levels to treat pain have taken several forms, but the goal is to inhibit the binding of NGF to TrkA on the nociceptive neurons by using either competitive antagonists, or antibodies to NGF or to the binding domain of TrkA (Watson et al., 2008).

1. TrkA-Fc, the ligand binding domain of TrkA bound to the Fc portion of human IgG, yields a soluble molecule that binds NGF with high affinity and sequesters the ligand to prevent binding to TrkA on nociceptive neurons. Infusion of this TrkA-Fc prevented the pain response in animal models of inflammation.
2. Anti-NGF antibodies have been efficacious in reducing pain in a variety of animal models, including peripheral nerve injury, partial spinal cord transection and bone pain associated with fracture or an animal model of cancer. The humanized version of some of these antibodies are in clinical trials for treatment of various types of inflammatory and chronic pain (Cattaneo, 2010).
3. TrkAd5 is a small portion of TrkA that contains the NGF-binding domain and can be produced as a small soluble protein that sequesters NGF with high affinity. Several models of inflammation have demonstrated the efficacy of this therapeutic agent in preventing hyperalgesia.

These approaches focus on sequestering NGF to prevent binding to TrkA. Additional approaches focus on blocking the receptor, either with antibodies or antagonists. A monoclonal antibody to TrkA (MNAC13) has been developed that blocks the NGF binding site on the receptor (Ugolini et al., 2007). All the antibody approaches have drawbacks associated with the size of the molecule potentially limiting its availability, as well as the possibility of generating an immune response. The development of small molecule antagonists is an alternative approach to prevent the binding of NGF to TrkA. All of the strategies discussed have been tested in animal models and show efficacy in reducing hyperalgesia in multiple paradigms of inflammatory and neuropathic pain. Several of these approaches are currently in clinical development for therapeutic use (see also Pain in Chap. 54).

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Alzheimer's disease. However, all these neuronal populations can also be supported by other factors as well, and understanding the complexity of these growth factor interactions and whether they can have therapeutic application remains a challenge.

Recent studies on BDNF in the brain have demonstrated that this factor influences many facets of neuronal development and function, including differentiation, dendritic growth and arborization, and synaptogenesis as well as neuronal survival. Synaptic activity, in particular glutamate release from presynaptic terminals, also regulates the synthesis and release of BDNF from the postsynaptic neurons, which in turn can induce phosphorylation of glutamate receptor subunits, enhancing the response to the neurotransmitter. This bidirectional communication between the presynaptic neurotransmitter regulating the postsynaptic release of BDNF can result in synapse stabilization and improved efficacy of neurotransmission. BDNF can also influence physiological function of mature neurons, regulating synaptic plasticity of hippocampal neurons and thereby affecting the ability to alter synaptic strength in response to experience. These effects on long-term potentiation (LTP) suggest that regulation of BDNF has implications for a role in a variety of cognitive functions.

While it would be very useful to study mice lacking BDNF or the TrkB receptor in the adult for effects on these neural functions, knockout of the BDNF or TrkB gene results in early postnatal death (Ernfors et al., 1995; Klein et al., 1993). Development of conditional knockouts of the TrkB gene in specific cell populations at specific development times has allowed a more detailed analysis of the role of BDNF on neuronal function. Additional genetic models, described below, have facilitated the analysis of the role of BDNF in cognitive function.

BDNF plays a role in the regulation of a number of psychiatric disorders including cognitive deficits, anxiety and eating disorders. A frequently occurring natural polymorphism has been found in the human BDNF gene that substitutes a methionine for a valine at position 66 of the pro domain of proBDNF and results in decreased secretion of BDNF. Among the behavioral consequences of this genetic polymorphism is a decrease in cognitive abilities that are dependent on hippocampal function. Individuals with this polymorphism have smaller hippocampi, possibly because of decreased neuronal survival during development, and deficits in dendritic arborization. The impairment in cognitive function may be due to the decreased size of the hippocampus as well as to a deficiency in the effects of BDNF on synaptic function in the adult. Brain imaging by fMRI has demonstrated abnormal patterns of activation during cognitive tasks, with reduced hippocampal activity. A mouse model of the BDNF val66met polymorphism demonstrated that in addition to the cognitive deficits, there was an increase in anxiety behavior when exposed to stressful situations, revealed in an inability to extinguish learned fear (Chen et al., 2008). Several studies have now shown that both acute and chronic stress lead to increased BDNF levels, and a reduced level of available BDNF adversely affects the ability to cope with stress appropriately. This val66met polymorphism in the BDNF gene has also been associated with depression, and may be considered a risk factor. Decreased levels of BDNF have been found in

individuals suffering from depression, and administration of BDNF can have antidepressant effects. Thus, BDNF can influence a broad spectrum of neuronal functions with psychiatric consequences.

Neurotrophin 3

Once NGF and BDNF were purified and cloned, their similarities suggested that they might be part of a family of related proteins, and additional members were identified. Molecular cloning led to the identification of two additional members of this family, neurotrophin-3 (NT3) and neurotrophin-4 (NT4). In the dorsal root ganglia (DRG), the large proprioceptive neurons depend on NT3 for survival. These neurons are dependent on NT3 even before they reach their targets in the muscle spindles. NT3 is expressed in the peripheral tissues surrounding the developing growth cone, and later is produced by the target muscle after innervation has occurred. During embryonic development, many neuronal progenitor populations are dependent on NT3, including migrating neural crest cells that give rise to the enteric nervous system as well as sympathetic and sensory ganglia. While some of these neuronal populations, such as the DRG proprioceptive neurons and cochlear sensory neurons in the ear, retain their dependence on NT3 in the adult, other populations switch their trophic dependence. For example, sympathetic neurons require NT3 during early embryonic development, and then become dependent on NGF as they reach their target. Thus, during development, the requirement of specific neuronal populations for particular factors can change with maturity.

Analysis of NT3^{-/-} mice demonstrated a loss of the large proprioceptive sensory neurons, as well as the muscle spindles to which they project. However, these mice actually showed a much greater loss of sensory neurons than just the proprioceptive population, demonstrating the absolute need for many different subpopulations for NT3 early in development, before switching their trophic dependence to another factor.

In the CNS, NT-3 and its TrkC receptor are widely expressed, although the function of this neurotrophin has not been as well characterized as NGF and BDNF. NT-3 can rescue survival of locus noradrenergic neurons after injury and promote development of hippocampal and cerebellar granule neurons. This neurotrophin can also contribute to motor neuron survival, one of many factors that can affect this neuronal population. In addition, NT-3 is critical for proper oligodendrocyte development, and mice lacking NT-3 or its TrkC receptor are deficient in cells of the oligodendrocyte lineage. (Barres et al., 1994)

Neurotrophin 4

Neurotrophin 4 is the least studied member of the neurotrophin family. Genetic knockout of NT4 does not result in loss of any DRG sensory neurons, although this factor does influence survival of spinal motor neurons. NT4 is expressed in muscle, and its expression is regulated by activity of innervating motor neurons, which increase its production, thereby providing trophic support to those innervating neurons. This provides an excellent example of bidirectional communication

between the target and the afferent neurons, in which the activity of the innervating neurons and the release of trophic factor from the target influence each other to fine-tune the synaptic connections.

REGULATION OF NEUROTROPHIN EXPRESSION

Neurotrophin genes contain the protein coding domains in a single 3' exon, with multiple 5' exons that generate transcripts that are alternatively spliced and differentially regulated. The BDNF gene has been the most extensively studied and has the most complex structure, with multiple different promoters and two alternative polyadenylation sites that generate either a long or a short 3' untranslated region (UTR). All of the possible transcripts generated from the different promoters with the different 3' UTRs encode the same BDNF protein, but the untranslated regions determine where and how much BDNF is produced. The BDNF transcripts with the long 3' UTR are targeted into dendrites, where BDNF protein can be locally translated in response to neuronal activity. The different 5' exons have distinct regulatory sequences, such that some exons direct constitutive BDNF expression, while others are regulated by neuronal activity, and allow expression of BDNF to be modulated by the level of activity. This regulation facilitates the coordination of experience-driven neural activity with the production of a neurotrophic factor that can facilitate stabilization of neuronal circuits.

PRONEUROTROPHINS

Neurotrophins are synthesized as larger precursor proteins, the proneurotrophins, which may be cleaved to generate the mature peptide. The classical understanding of NGF synthesis is that the precursor, proNGF, is cleaved intracellularly by the enzyme furin to generate the mature NGF peptide. However, proneurotrophins can also be secreted without being cleaved. The secreted proneurotrophins have different receptor affinities and different functions than the cleaved, mature neurotrophins (Lee et al., 2001). Mature neurotrophins elicit their trophic actions via their respective Trk receptors, but proneurotrophins appear to act through p75NTR. While all the neurotrophins can also bind to the p75NTR with low affinity, recent studies have determined that the proneurotrophins can bind p75NTR with high affinity. This is due to the binding of the proneurotrophin to a receptor complex composed of p75NTR and sortilin (Figure 29-3), which can induce cell death, in contrast to the survival signaling, promoted by mature neurotrophins via Trk receptor signaling. The regulatory mechanisms that determine whether a proneurotrophin is cleaved intracellularly or secreted without being cleaved are currently unknown, but clearly of great functional importance, since the release of a proneurotrophin may bind the p75-sortilin receptor complex and elicit neuronal cell death, while release of cleaved neurotrophins can bind a p75-Trk receptor complex and promote neuronal survival (Chao & Bothwell, 2002).

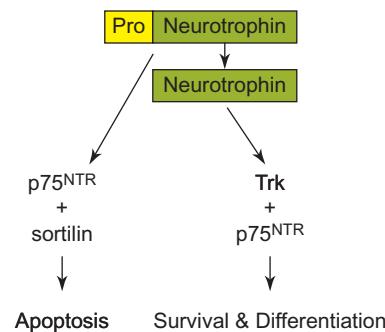


FIGURE 29-3 Cleavage of proneurotrophins. Proneurotrophins bind to a receptor complex composed of p75NTR and sortilin, and can induce apoptosis. Mature, cleaved neurotrophins bind to a complex composed of a Trk receptor with p75NTR to promote survival and differentiation.

ProNGF secretion has been detected in the CNS primarily after injuries, conditions in which p75NTR is also upregulated. The increase in proNGF secretion and p75NTR expression leads to an environment that is more vulnerable to neuronal death, and appears to be responsible for at least some neuronal loss after different types of insults (Friedman, 2010).

Just as the interaction of neurotrophins with Trk receptors regulates more than just cell survival, the effect of proneurotrophins on p75NTR does not only regulate cell death. In the hippocampus proBDNF is synthesized and can be released in the precursor form. Once secreted, proBDNF can be cleaved extracellularly to generate BDNF, which binds TrkB and can influence synaptic activity by facilitating long-term potentiation (LTP). Alternatively, uncleaved proBDNF can bind p75NTR and facilitate long-term depression (LTD), another form of synaptic plasticity. Therefore, the regulation of proBDNF cleavage, even after secretion, may have profound effects on synaptic activity in the hippocampus.

NEUROTROPHIN RECEPTORS

Neurotrophins can bind to two distinct types of receptors: members of the Trk receptor tyrosine kinase family, and the p75NTR (neurotrophin receptor). Multiple signaling pathways are activated by these receptors upon ligand binding. Three major signaling pathways in particular have been well characterized for the Trk receptors (Kaplan & Miller, 1997; Huang & Reichardt, 2003): activation of phosphoinositide 3-kinase (PI₃K) leading to phosphorylation of Akt, a critical protein in the regulation of cell survival; activation of the ras-MAP kinase pathway, which plays an important role in neuronal differentiation as well as survival; and activation of phospholipase C (PLC) γ , which regulates the recruitment of calcium and plays an important role in neuronal activity (Figure 29-4). In contrast, the p75NTR has no kinase activity and signals by recruiting intracellular binding proteins upon interaction with ligand. These intracellular binding proteins initiate a cascade of signaling events that regulate a variety of cellular functions.

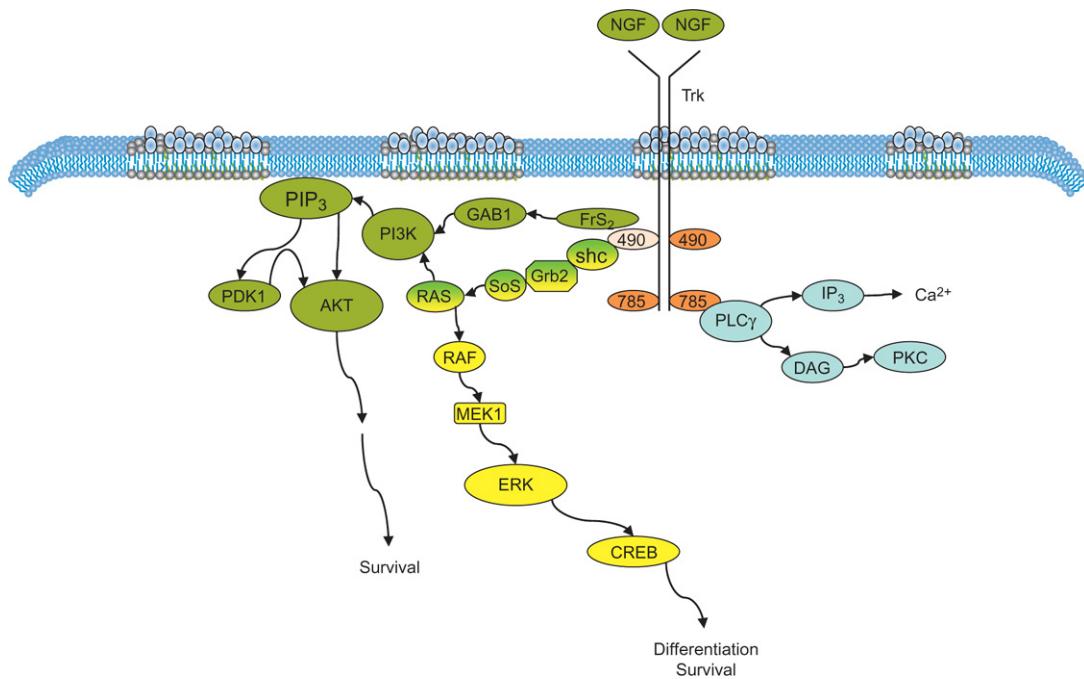


FIGURE 29-4 Trk receptor signaling. Signaling by Trk receptors activates three well-characterized pathways, the PI₃K-Akt pathway, the ras-MAPK pathway and recruitment of PLC γ .

Trk receptors

Trk receptors have intrinsic kinase activity, and dimerize upon the binding of the dimeric neurotrophin ligand. Three Trk receptors have been identified: TrkA, B and C. TrkA specifically interacts with NGF, while TrkB binds to both BDNF and NT-4. TrkC is the specific receptor for NT-3 (Figure 29-2). Binding of the neurotrophins to their respective Trk receptors is necessary to mediate the effects of these factors on neuronal survival and differentiation.

Dimerization of receptor tyrosine kinases causes them to become autophosphorylated on specific tyrosine residues in the intracellular domain. These phosphorylated tyrosine residues serve as docking sites for adapter proteins that allow additional kinases to be recruited, activating the pathways described above. The recruitment of PI₃K to the receptor allows the phosphorylation of the membrane lipid PIP₂ to form PIP₃, which leads to the downstream phosphorylation of the serine-threonine kinase Akt. Activation of Akt stimulates a cascade of events leading to the activation of pro-survival proteins and the suppression of pro-apoptotic proteins, thereby promoting neuronal survival.

Another major pathway activated by Trk receptors is the mitogen activated protein kinase (MAPK) pathway. When the Trk receptors dimerize, a series of adapter proteins leads to the recruitment of the small G protein ras, which activates the MAPK cascade leading to activation of ERK. ERK phosphorylates and activates transcription factors such as CREB, which regulates the expression of numerous genes involved in neuronal differentiation.

Expression of the different Trk receptors in specific cellular populations defines those neurons that are able to respond to

the neurotrophins. Consistent with the expression of NGF in the hippocampus and cortex to support the innervating cholinergic afferents from the basal forebrain, TrkA is principally found in those basal forebrain cholinergic neurons. In contrast, BDNF affects many neuronal populations in the brain, influencing dendritic growth and complexity and regulating a variety of behaviors as discussed above. Consistent with these more global effects, TrkB is localized throughout the hippocampus and cortex, as well as other neuronal populations that respond to BDNF or NT4 such as the substantia nigra and the motor neurons of the spinal cord.

The p75 neurotrophin receptor (p75NTR)

The p75NTR was actually the first receptor identified for NGF, and was initially characterized as a low-affinity receptor for all the neurotrophins. The absence of kinase activity in this receptor kept its functional importance obscure for many years, however recent studies have determined that the p75NTR can actually function as a death receptor. This creates an interesting situation where the two different neurotrophin receptors elicit opposing effects on neuronal survival. In many cases, the p75NTR functions in a receptor complex as a co-receptor. The initial studies on NGF binding demonstrated that the high-affinity-binding site was actually generated by a complex of TrkA and p75NTR, and that the presence of p75NTR enhanced ligand discrimination as well as binding affinity (Hempstead et al., 1991). However, in the absence of a Trk receptor, neurotrophins have been shown to induce cell death via the p75NTR. More recent studies have shown that proneurotrophins, which don't bind Trk, bind with high affinity to p75NTR and can elicit cell death even in the presence of Trk receptors.

Since p75NTR has no enzymatic activity, its signaling requires the recruitment of intracellular binding proteins. Several p75NTR-binding proteins have been identified that link the receptor to the downstream apoptotic machinery including promoting the release of cytochrome c from mitochondria and activation of the intrinsic caspase cascade (Figure 29-5). The p75NTR is widely expressed throughout the CNS during development, however it is developmentally down-regulated and in the adult is found in only a few neuronal populations in the brain. While the role of p75NTR in promoting apoptosis is the most well-characterized function for this receptor, it has been shown to play a role in other cellular functions as well, including neurite outgrowth, cell cycle regulation, and myelinization by Schwann cells, as well as regulating LTD in hippocampal neurons (Gentry et al., 2004). Clearly the function of this receptor is profoundly influenced by its cellular context.

What might be the purpose of having a receptor that supports neuronal survival in response to neurotrophins, and another receptor that elicits neuronal death in response to pro-neurotrophins? The neurotrophins binding to Trk receptors are critical for maintaining survival at various stages of development, and also for regulating neuronal function in the adult. The conditions in which proneurotrophins have been shown to induce apoptosis via p75NTR generally occur in injury or disease, conditions that are known to increase expression of p75NTR. Thus, it may be that the factors present during normal development promote survival and physiological function of the nervous system, but under pathological conditions, increases in proneurotrophins and expression of p75NTR tilt the balance of trophic factor signaling towards cell death.

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF)

The GDNF family ligands (GFLs) composed of GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN), can influence the survival and function of peripheral and central neurons (Figure 29-6). GDNF was first identified as a neurotrophic factor for substantia nigra dopaminergic neurons, and was subsequently shown to promote survival of spinal motor neurons, locus coeruleus noradrenergic neurons, and several other populations of CNS neurons. In fact, GDNF is more potent than neurotrophins in promoting survival of substantia nigra, locus coeruleus, and spinal motor neurons in culture. As with the neurotrophins, elimination of Ret, the signaling component of the GDNF receptor, specifically from dopaminergic neurons did not result in loss of these neurons, although expression of constitutively active Ret increased the number of dopaminergic neurons, suggesting that while these neurons respond to GDNF, their survival can be supported by multiple factors, and the removal of any one of these factors does not eliminate the population of cells. Due to its effects promoting survival of substantia nigra dopaminergic neurons, GDNF has been considered for possible therapeutic application in Parkinson's disease (PD). In both rodent and primate models of PD, GDNF was neuroprotective against toxin-induced neurodegeneration, however its efficacy in treating the human disease is still in question (Rangasamy et al., 2010). Moreover, a major challenge for the therapeutic use of any growth factor in treating neurodegenerative

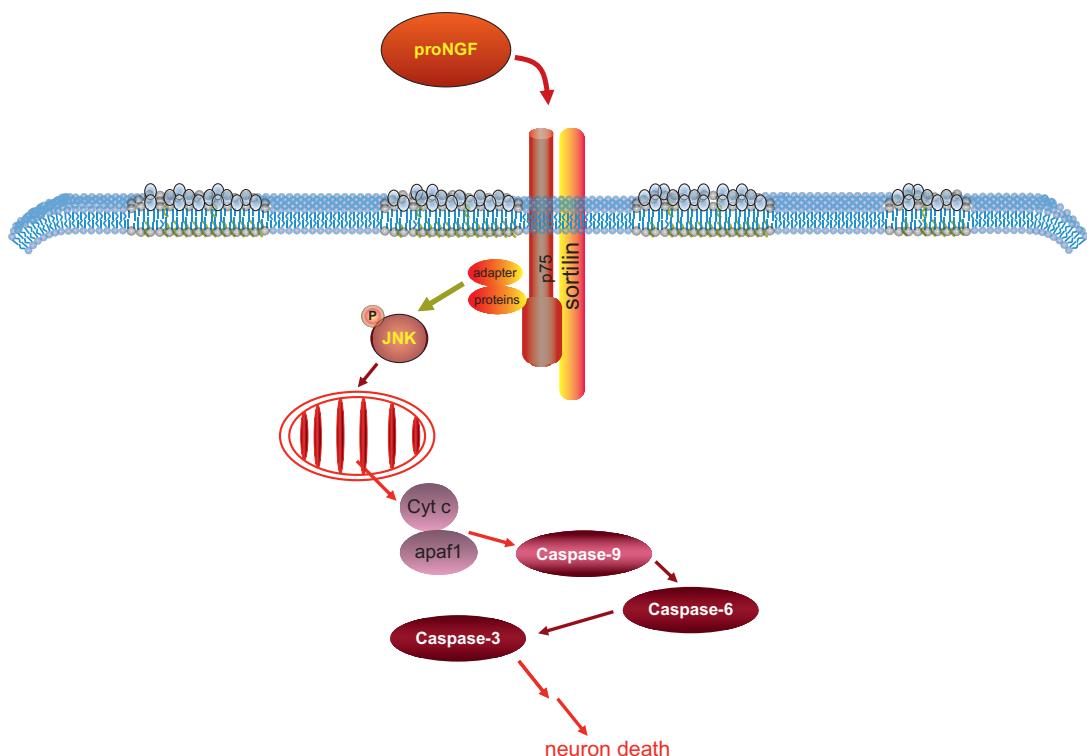


FIGURE 29-5 p75NTR signaling. Activation of p75NTR recruits adapter proteins to the receptor, leading to JNK phosphorylation, mitochondrial release of cytochrome c, and activation of the intrinsic caspase cascade.

diseases lies in the ability to deliver the factor to the necessary location in the brain, due to the inability of the proteins to cross the blood-brain barrier and to diffuse extensively within the brain parenchyma.

The second member of this growth factor family, neurturin, is similarly expressed in the substantia nigra and can promote survival of dopaminergic neurons in culture, and after damage induced by 6-hydroxydopamine treatment *in vivo* (Horger et al., 1998). This protein has also been developed for possible therapeutic treatment of PD, and has had some efficacy in animal models (Rangasamy et al., 2010).

In the peripheral nervous system, the GDNF family ligands can also promote survival of sympathetic and parasympathetic neurons. GDNF is expressed in peripheral tissues, consistent with a role as a target-derived trophic factor for sympathetic, parasympathetic, and sensory neurons. It is also synthesized by muscle tissue and retrogradely transported by spinal motor neurons, for which it is a potent survival factor. In addition, GDNF is essential for development of the enteric nervous system. It stimulates proliferation and migration of enteric progenitors and supports the survival of these neurons in the colon.

GFL RECEPTORS

The major signaling receptor for GFLs is the Ret receptor tyrosine kinase, but these ligands do not bind directly to this receptor; instead they bind to a GPI-linked receptor known as GDNF family receptor α , or GFR α . There are four members of the GFR α family, and they bind preferentially to the

different members of the GDNF family. GDNF binds GFR α 1, NRTN binds GFR α 2, ARTN binds GFR α 3 and PSPN interacts with GFR α 4. The binding of the GDNF family ligands to their respective GFR α allows them to activate signaling through the Ret receptor (Airaksinen & Saarma, 2002). It should be noted that Ret is also expressed outside the nervous system, and mice lacking this receptor die shortly after birth due to major defects in kidney development. These mice also show a severe deficit in enteric neurons, which, like other peripheral neuronal populations, are derived from the neural crest. Mutations in Ret lead to Hirschsprung's disease, a condition characterized by the absence of enteric neurons in the distal part of the gut. Mice lacking GDNF or GFR α 1 show deficits similar to the Ret-/- mice, indicating that the GDNF ligand acts via a receptor complex composed of GFR α 1 and Ret. The signaling downstream of Ret activates pathways similar to other receptor tyrosine kinases such as the Trk receptors, which include activation of PI₃K-Akt, ras-MAPK and PLC γ .

GDNF also can promote survival of sympathetic and sensory neurons, indicating some functional overlap with members of the neurotrophin family of factors. In some populations there may be sequential dependence on different trophic factors. The small nociceptive DRG sensory neurons that express TrkA and depend on NGF during embryonic development become NGF-independent postnatally, although many continue to express TrkA and respond to NGF functionally. However, a subpopulation of these neurons downregulate TrkA and induce Ret expression. These neurons become dependent on GDNF, indicating that some neurons switch their trophic dependence with maturation.

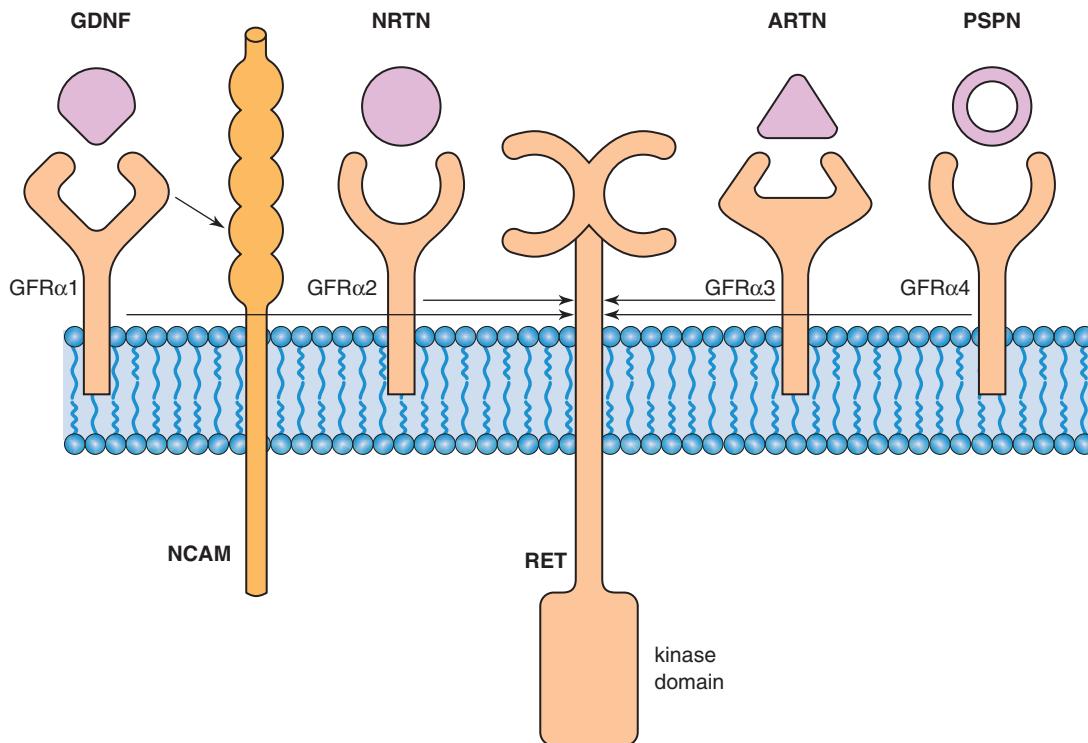


FIGURE 29-6 GDNF family and receptors. Each GDNF family ligand binds its respective GFR α in order to activate signaling via the Ret receptor. Alternatively, GDNF and GFR α 1 can activate signaling via NCAM.

Since the GFLs signal through a receptor complex that contains a GFR α and Ret, it is interesting to note that expression of the GFR α receptors can also be found in cells that do not express Ret, suggesting that binding of GFLs to their respective GFR α s may sometimes act in a Ret-independent manner and recruit other signaling proteins. In fact, GDNF bound to GFR α 1 can associate with the neural cell adhesion molecule NCAM, which downregulates its adhesive character and activates signaling through the Src family kinase Fyn (Paratcha & Ledda, 2008). Several functional outcomes of this association between GDNF-GFR α 1 and NCAM have been characterized, including regulation of Schwann cell migration in peripheral nerves and migration of olfactory neuron progenitors in the rostral migratory stream, as well as neurite outgrowth of cortical and hippocampal neurons (Paratcha & Ledda, 2008).

In addition to these established mechanisms of GFL signaling by interacting with GFR α receptors and recruiting either Ret or NCAM, additional functions of GDNF have been described that do not require either of these signaling mechanisms. GDNF has been shown to regulate the differentiation and migration of cortical GABAergic neurons, which express neither Ret nor NCAM. These studies suggest that there may be yet other signaling receptors for this family of neurotrophic factors (Pozas & Ibanez, 2005), demonstrating the complexity of the GDNF family of factors in their ability to associate with their respective GFR α s, and subsequently interact with multiple different signaling receptors for distinct functional purposes.

NEUREGULINS

The neuregulins (NRG) are a family of factors that play important roles in the interactions between neurons and glia (see Ch. 28). The Nrg1 gene gives rise to multiple different isoforms generated from the use of alternate promoters and differential splicing. These isoforms are divided into three major families referred to as neuregulin 1 types I, II or III. Neuregulin 1 type I was originally known as neu differentiation factor, acetylcholine receptor-inducing activity, or heregulin. This protein lacks a signal peptide, but is synthesized as a transmembrane protein that is proteolytically cleaved to release the growth factor. The type II isoform, originally known as glial growth factor (GGF), is a secreted protein, while type III Nrg1 remains tethered to the membrane, and therefore requires cell-cell contact for its trophic activity. This isoform is primarily expressed in neurons. Two additional neuregulin genes, Nrg2 and Nrg3, are expressed in the nervous system, but their functions are largely unknown.

The Nrg1 proteins all contain an EGF domain that is necessary for receptor binding. These factors bind to the ErbB family of receptors that are related to the EGF receptor (also known as ErbB1). The Nrg1 ligands bind to ErbB3 or ErbB4. ErbB3 lacks a kinase domain, and therefore in order to transduce a signal it forms a heterodimer with ErbB2, which has a kinase domain but cannot bind ligand.

Neuregulins are among the many growth factors that regulate migration of neural crest cells during embryonic development. These migratory cells express ErbB receptors, and various isoforms of Nrg1 are produced along the migratory route. Of the neural crest derivatives that require neuregulins,

Schwann cells have been extensively studied. Schwann cells express the ErbB2-ErbB3 receptor complex, and Nrg1 regulates all aspects of Schwann cell development, including migration, proliferation, differentiation and myelination (see Ch. 31). The regulation of peripheral myelination is one of the most well-characterized functions of type III Nrg1 (Nave & Salzer, 2006). This Nrg1 isoform is expressed by peripheral axons where it remains tethered to the membrane, and the level of Nrg1 type III expressed on the peripheral axons directly influences the degree of myelination. Overexpression of Nrg1 type III in small sensory neurons or sympathetic neurons that are normally unmyelinated will induce them to be myelinated, and the absence of Nrg1 type III results in the absence of myelin on sensory neurons that are normally myelinated. An absence of ErbB3 not only shows a severe deficit in Schwann cells, but also a significant loss of associated motor neurons, confirming that trophic support from surrounding glia is important for axonal maintenance and growth prior to target innervation. While continued ErbB signaling is not necessary for myelin maintenance, activation of this receptor in the mature nerve also mediates the Schwann cell response to peripheral nerve injury and can initiate a demyelination process. Thus, Nrg1 type III can initiate myelination in the immature peripheral nerve, or demyelination after injury to the nerve.

In the brain, Nrg1 can influence many aspects of neuronal development, including neuronal migration, and regulation of neurotransmitter receptors for acetylcholine, glutamate and GABA. Polymorphisms in the Nrg1 gene have been associated with several psychiatric disorders including schizophrenia, which is thought to be affected by these neurodevelopmental events. Altered levels of Nrg1 expression may lead to deficits in neuronal migration and thereby affect establishment of appropriate connectivity, and may also alter the levels of neurotransmitter receptors, leading to deficient neurotransmission, also considered a contributing factor to schizophrenia (Corfas et al., 2004).

Cells of the oligodendrocyte lineage also express ErbB receptors, and Nrg1 can regulate oligodendrocyte development, promoting survival and differentiation of oligodendrocyte progenitors. The role of Nrg1 in directly promoting myelination of CNS axons by oligodendrocytes is less well understood than its role in peripheral myelination by Schwann cells, however the Nrg1 effects on oligodendrocyte development has consequences for proper myelin formation in the brain. Interestingly, recent studies have demonstrated myelin alterations in schizophrenic patients, adding to the evidence for a possible role of Nrg1 as a risk factor for this devastating disease (Corfas et al., 2004).

NEUROTROPHIC CYTOKINES

A family of factors related to the immune cytokine interleukin-6 (IL-6) has also been shown to have neurotrophic activity. While IL-6 itself is not abundant in the nervous system and does not have well-defined neurotrophic effects, the related factors leukemia inhibitory Factor (LIF), ciliary neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) have clearly established trophic effects on specific neuronal populations. These neurotrophic cytokines signal through a common receptor subunit, gp130. While IL-6 signals through a gp130

homodimer, LIF, CNTF and CT-1 signal through a heteromeric complex composed of gp130 and LIF receptor β (LIFR β). While LIF and CT-1 bind directly to the gp130-LIFR β complex, CNTF initially binds to a distinct alpha subunit, which can form a tripartite receptor complex with LIFR β and gp130. Activation of this receptor complex recruits members of the JAK family of cytoplasmic tyrosine kinases for signaling. Activation of Jak kinases leads to recruitment and activation of STAT proteins, which can then translocate to the nucleus to regulate gene expression.

CNTF is expressed in glial cells of the central and peripheral nervous systems, and was initially identified for its ability to promote survival of parasympathetic ciliary neurons, as the name implies. Subsequently it was shown to have a broader spectrum of trophic activities, supporting a variety of sensory and sympathetic neurons in addition to the parasympathetic neurons. CNTF also has effects on neuronal populations in the CNS, including hippocampal neurons, basal forebrain neurons and particularly motor neurons. CNTF is synthesized in muscle and Schwann cells, and can protect motor neurons from death after injury and in animal models of motor neuron disease.

Motor neurons in mice lacking CNTF develop normally, and this is likely due to functional compensation from LIF and/or CT-1, since they signal through the same receptor complex. Triple knockout of these neurotrophic factors revealed significant deficits in motor neuron numbers and motor function. These studies revealed that CNTF and LIF primarily support motor neurons postnatally, while CT-1 promotes survival of these neurons prenatally (Holtmann et al., 2005). Despite the importance of these factors for motor neuron development and maintenance, genetic deletion of all three factors did not lead to a total loss of motor neuron survival and function, confirming that many different factors can support motor neuron survival and function as mentioned previously, including BDNF, NT4, GDNF, and many others.

Several instances of glial-derived trophic factors have been described here that regulate neuronal survival and function, such as the production of CNTF by Schwann cells to support motor neurons. However, the reverse situation can also be true—that neurons produce factors to regulate glial development and function. During embryonic development, neurons are produced first by progenitors in the ventricular zone, followed by the generation of astrocytes and oligodendrocytes. Once cortical neurons are generated, they produce CT-1, which then promotes the progenitors to generate astrocytes. If CT-1 is provided to the cortical progenitors during early embryogenesis during the time that only neurons are usually being produced, astrocytes will be generated prematurely. Conversely, an absence of CT-1 results in deficient astrogliogenesis (Barnabe-Heider et al., 2005). Therefore, neurotrophic factors can mediate the development and function of both neurons and glia.

The families of factors discussed above have their primary actions in the nervous system, but other factors that were initially discovered in other systems also affect neuronal and glial development and function. Many of these factors have important functions in the nervous system during early development, similar to their functions in other systems, and are discussed in Chapter 28. For example, the fibroblast growth factors (FGFs) were characterized for their ability to promote fibroblast proliferation, and have also been shown to promote

TABLE 29-1 Growth Factors Acting in the Nervous System

| Growth Factor | Receptor |
|--|---------------------------------------|
| Neurotrophins | |
| Nerve growth factor | TrkA |
| Brain-derived neurotrophic factor | TrkB |
| Neurotrophin 3 | TrkC |
| Neurotrophin 4/5 | TrkB |
| Neurokines | |
| Ciliary neurotrophic factor | CNTFR α , LIFR β + gp130 |
| Leukemia inhibitory factor | LIFR β , gp130 |
| IL6 | IL6R α , gp130 |
| Cardiotrophin 1 | LIFR β , gp130 + ? |
| Fibroblast growth factors | |
| FGF-1 (acidic FGF) | FGFR1–4 |
| FGF-2 (basic FGF) | FGFR1–3 |
| Transforming growth factor β superfamily | |
| Transforming growth factors β | TGF β RI and TGF β RII |
| Bone morphogenetic factors | BMPR I and BMPRII |
| Glial-derived neurotrophic factor family | |
| Glial-derived growth factor | c-Ret, GDNFR α -1 |
| Neurturin | c-Ret, GDNFR α -2 |
| Artemin | c-Ret, GDNFR α -3 |
| Persephin | c-Ret, GDNFR α -4 |
| Epidermal growth factor superfamily | |
| Epidermal growth factor | EGFR |
| Transforming growth factor α | EGFR |
| Neuregulins [GGF, ARIA, SMDF, etc.] | ErbB2, ErbB3, ErbB4 |
| Other growth factors | |
| Platelet derived growth factor | PDGFR α and β |
| Insulin-like growth factor I | IGFR-I |
| Hepatocyte growth factor | c-Met |
| Macrophage-stimulating protein | c-Ron |

proliferation of neuronal and glial progenitors. There are four FGF receptors, three of which are expressed in the nervous system. The family of bone morphogenetic proteins (BMPs) a subclass of the TGF β family of factors, is important for tissue morphogenesis, and plays a critical role in the initial specification of the neural ectoderm, which actually requires repression of BMP signaling. BMPs also play a role in the formation of the neural crest, and subsequently in the migration and

differentiation of these precursor cells into specific types of neurons. BMPs, acting together with other proteins, including the Wnt family of factors and sonic hedgehog (SHH), are crucial for patterning of the nervous system during development, but are also involved in morphogenesis of other tissues. All these factors also have trophic effects on different populations of neurons at later stages of development.

It is not the purpose of this chapter to provide an exhaustive list of all the growth factors that function in the nervous system. Many are summarized in Table 29-1.

SUMMARY AND CONCLUSIONS

Every aspect of cellular function can be influenced by growth factors, including survival, proliferation, migration, and differentiation. The nervous system is composed of such a heterogeneous population of cells that the specific trophic requirements for appropriate development and function are extremely complex. Although the original studies on trophic factors defined their function in terms of their ability to support survival of different populations of neurons, recent studies have shown that these factors regulate many aspects of neuronal function. Particularly in the CNS, where a population of neurons may respond to a plethora of different factors, understanding the role of each of these factors remains a significant challenge. Current and future studies will provide additional insights into the mechanisms of trophic interactions, and especially whether they may be harnessed for therapeutic purposes.

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