SUGGESTED CROSS REFERENCES

Please verify cross references. The cross references provided above seemed to be out of date and have been updated to correspond to the table of contents of the most recent edition. Please verify that the update cross references are correct.

Section 1.11 discusses basic molecular neurobiology, section 1.12 discusses psychoneuroendocrinology, and neuropsychiatric aspects of endocrine disorders are discussed in section 24.7.

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▲ 1.7 Neurotrophic Factors

FRANCIS S. LEE, M.D., PH.D., AND MOSES V. CHAO, PH.D.

Neurotrophins are a unique family of polypeptide growth factors that influence the proliferation, differentiation, survival, and death of neuronal and nonneuronal cells. These proteins emerged initially in vertebrate species and do not exist in invertebrates such as *Drosophila melanogaster* or *Caenorhabditis elegans*. This late evolution of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, and NT-4 as a family implies that these signaling molecules may act to mediate additional higher-order activities, such as learning, memory, and behavior, in addition to their established functions for cell survival. The effects of neurotrophins depend upon their level of availability, their affinity of binding to transmembrane receptors, and the downstream signaling cascades that are stimulated after receptor activation. Neurotrophins play multiple roles in the adult nervous system: Regulating synaptic connections and synapse structure, neurotransmitter release and potentiation, mechanosensation, and pain and synaptic plasticity. Alterations in neurotrophin levels have been implicated in neurodegenerative disorders such as Alzheimer's disease and Huntington's disease, as well as psychiatric disorders such as depression and substance abuse. These new insights have important implications for the etiology and treatment of psychiatric disorders.

THE NEUROTROPHIN FAMILY

A large number of polypeptide factors affect the survival, growth, and differentiation of the nervous system. The neurotrophins, comprised of NGF, BDNF, NT-3, and NT-4, are best understood and most widely expressed in the nervous system. The neurotrophins are initially synthesized as precursors or proneurotrophins that are cleaved to release the mature, active proteins. The mature proteins, approximately 12 to14 kDa in size, form stable, noncovalent dimers and are normally expressed at very low levels during development. Proneurotrophins are cleaved intracellularly by furin or proconvertases utilizing a highly conserved dibasic amino acid cleavage site to release C-terminal mature proteins. The mature proteins mediate neurotrophin actions by selectively binding to members of the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases to regulate neuronal survival, differentiation, and synaptic plasticity. In addition, all mature neurotrophins interact with $p75^{NTR}$, which can modulate the affinity of Trk neurotrophin associations.

NGF was the first identified neurotrophic factor and has a restricted distribution within the neurotrophin family. In the peripheral nervous system (PNS), it acts on sympathetic neurons as well as sensory neurons involved in nociception and temperature sensation. In the central nervous system (CNS), NGF promotes the survival and functioning of cholinergic neurons in the basal forebrain. These neurons projects to the hippocampus and are believed to be important for memory processes, which are specifically affected in Alzheimer's disease. The other neurotrophins are more widely expressed in the CNS. BDNF and NT-3 are highly expressed in cortical and hippocampal structures and have been linked to the survival and functioning of multiple neuronal populations.

NEUROTROPHIN RECEPTORS

Neurotrophins are unique in exerting their cellular effects through the actions of two different receptors, the Trk receptor tyrosine kinase and the p75 neurotrophin receptor ($p75^{NTR}$), a member of the tumor necrosis factor (TNF) receptor superfamily. Trk receptors consist of an extracellular ligand-binding region, a single transmembrane domain, and a highly conserved intracellular tyrosine kinase domain. The $p75^{NTR}$ receptor consists of an extracellular ligand-binding region, a single transmembrane domain, and an intracellular portion containing a protein-association region termed the death domain (Fig. 1.7–1). All neurotrophins bind to the p75 receptor. There are three vertebrate *trk* receptor genes, *trkA*, *trkB*, and *trkC*. All Trk receptors exhibit high conservation in their intracellular domains, including the catalytic tyrosine kinase domain and the juxtamembrane domain. The Trk receptors also exhibit a number of truncated isoforms. There are no sequence similarities between Trk and p75 receptors in ~~their either~~ ligand-binding or cytoplasmic domains.

Fig. 1.7–1

Neurotrophins bind as dimers to Trk family members, leading to receptor dimerization and activation of the catalytic tyrosine protein kinase domains. The dimerized Trk receptors autophosphorylate several key intracellular tyrosine residues, which rapidly initiates intracellular signaling cascades. This is accomplished by the phosphorylated tyrosines on the receptor acting as recognition sites for the binding of specific adaptor proteins that contain phosphotyrosine-binding motifs such as Src homology domain 2 (SH2). In particular, the Shc adaptor protein links the activated Trk receptor to two separate

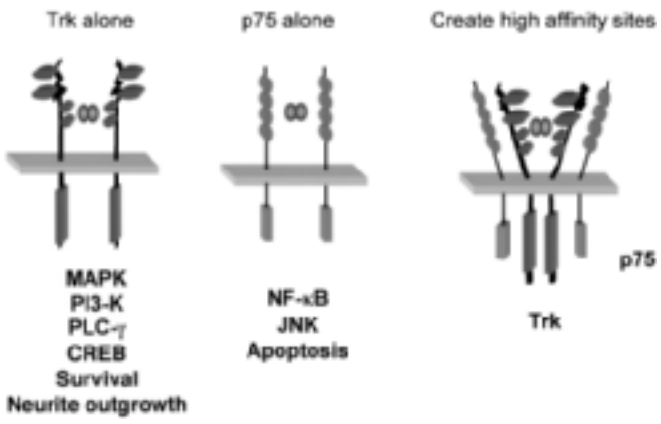

FIGURE 1.7–1. Neurotrophin receptor signaling. Neurotrophins bind to Trk tyrosine kinase receptors (*right*) and p75 neurotrophin receptors ($p75^{NTR}$) (*middle*). Trk receptors mediate differentiation and survival signaling through mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3-K), and phospholipase C-γ (PLC-γ) pathways, which lead to effects on transcription factors, such as the cyclic adenosine monophosphate response element binding protein (CREB). Trk receptors contain IgG domains for ligand binding and a catalytic tyrosine kinase sequence (*left*) in the intracellular domain. $p75^{NTR}$ mediates apoptotic and cell migration responses through nuclear factor κB (NF-κB) and c-Jun N-terminal kinase (JNK) pathways. The extracellular part of $p75^{NTR}$ contains four cysteine-rich repeats; the intracellular domain contains a death domain (*middle*). Interactions between Trk and $p75^{NTR}$ receptors can lead to changes in binding affinity for neurotrophin (*right*).

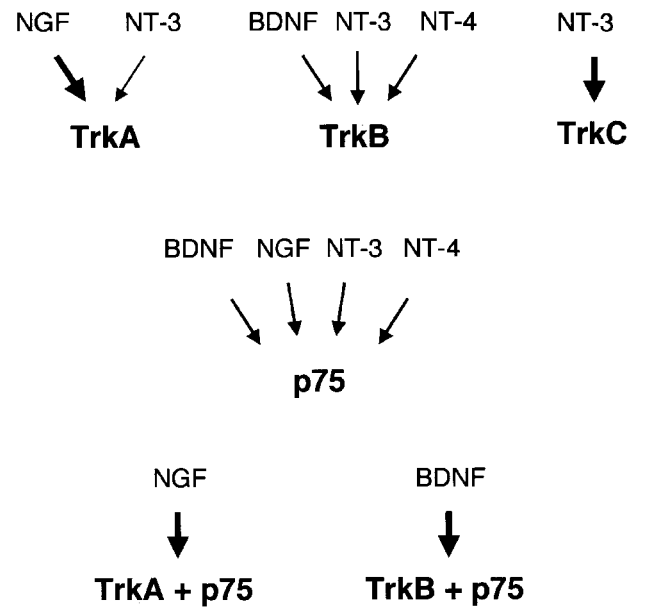

FIGURE 1.7–2. Neurotrophin binding specifities. All neurotrophins bind to p75 neurotrophin receptors ($p75^{NTR}$). Neurotrophins bind selectively to specific tropomyosin-related kinase (Trk) receptors, and this specificity can be altered by $p75^{NTR}$. Several neurotrophins, neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), can bind to multiple Trk receptors. BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor.

intracellular signaling pathways that mediate the majority of the biological effects of neurotrophins.

The primary survival pathway involves Shc linking Trk receptor activation to increases in phosphotidylinositol-3-kinase (PI3 kinase) activity. This in turn activates another protein kinase, Akt (protein kinase B), which has multiple effects on the cell's apoptotic pathways. Also, Shc phosphorylation by Trk receptor activation leads to increases in Ras and MAP kinase activities. These events in turn influence transcriptional events such as the induction of the CREB transcription factor. CREB produces a multitude of effects on the cell cycle, neurite outgrowth, and synaptic plasticity. In addition, phospholipase-C-γ (PLC-γ) binds to activated Trk receptors and initiates an intracellular signaling cascade release of inositol phosphates and activation of protein kinase C (PKC). Trk receptor activation leads to a multitude of downstream signaling events, leading to changes in transcriptional programs.

NGF binds most specifically to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC receptors. The $p75^{NTR}$ receptor can bind to each neurotrophin but has the additional capability of regulating a Trk's affinity for its cognate ligand. Trk and $p75^{NTR}$ receptors have been referred to as high- and low-affinity receptors, respectively. However, this is not correct since TrkA and TrkB actually bind mature neurotrophins with an affinity of 10^{-9} to 10^{-10} M, which is lower than the high-affinity site ($K_d = 10^{-11}$M). Also, the precursor form of NGF displays high-affinity binding to $p75^{NTR}$. Trk-mediated responsiveness to low concentrations of NGF is dependent upon the relative levels of $p75^{NTR}$ and TrkA receptors and their combined ability to form high-affinity sites. This is important since the ratio of receptors can determine responsiveness and ultimately neuronal cell numbers.

Although p75 and Trk receptors do not bind to each other directly, there is evidence that complexes form between the two receptors. Perhaps as a result of these interactions, increased ligand selectivity can be conferred onto Trk receptors by the p75 receptor. One way of generating specificity is by imparting greater discrimination of ligands for the Trk receptors (Fig. 1.7–2). For example, BDNF, NT-3, and NT-4/5 can each bind to the TrkB receptor, but in the presence of p75 only BDNF provides a functional response. Likewise, NGF and NT-3 both can bind to TrkA, but p75 restricts the signaling of TrkA to NGF and not to NT-3 (Fig. 1.7–2). Hence, p75 and Trk receptors interact in order to provide greater discrimination among different neurotrophins. **Fig. 1.7–2**

NEUROTROPHIC FACTORS AND DEVELOPMENT

The formation of the vertebrate nervous system is characterized by widespread programmed cell death, which determines cell number and appropriate target innervation during development. Neurotrophins are highly expressed during early development and have been shown to be essential for survival of selective populations of neurons during different developmental periods. The neurotrophic hypothesis provides a functional explanation for the role of neurotrophic factors in the development of the nervous system (Fig. 1.7–3). During development, neurons approaching the same final target vie for limited amounts of target-derived neurotrophic factors. In this way, the nervous system molds itself to maintain only the most competitive and appropriate connections. Competition among neurons for limiting amounts of neurotrophin molecules produced by target cells accounts for selective cell survival (Fig. 1.7–3). Two predictions emanate from this hypothesis. First, the efficacy of neuronal survival will depend upon the amounts of trophic factors produced during development. Second, specific receptor expression in responsive cell populations will dictate neuronal responsiveness. **Fig. 1.7–3**

On one level, neurotrophins fit well with the neurotrophic hypothesis, as many peripheral neuronal subpopulations depend on a specific neurotrophin during the period of naturally occurring cell death. In the CNS, the overlapping expression of multiple neurotrophin receptors

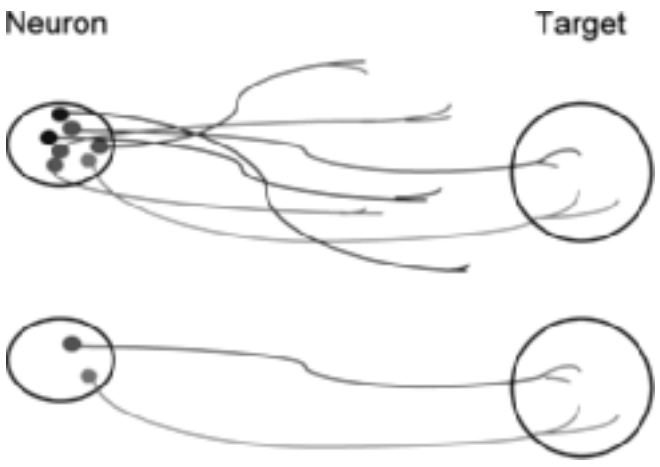

FIGURE 1.7–3. The neurotrophin hypothesis. Neurons compete for limited quantities of neurotrophins in target regions, which leads to selective neuronal survival. Levels of target-derived neurotrophins and neurotrophin receptors will determine efficacy of survival and responsiveness of the neurons. The ability to form high-affinity binding sites allows for greater responsiveness under limiting quantities of trophic factors. Lack of trophic support or incorrect targeting of axons to the wrong target results in programmed cell death.

and their cognate ligands allows for more diverse connectivity, which extends well into adulthood. In addition, it is clear now that neurons can release neurotrophins that act on themselves (autocrine transmission) or can be anterogradely transported down axons and act on neighboring neurons. Also, glial cells can release neurotrophins that act upon neurons in a paracrine fashion. In the periphery, neurotrophin retrograde signaling occurs through a pathway that must efficiently transmit information over long distances, at times over a meter.

Neurotrophins promote cell survival and differentiation during neural development. Paradoxically, they can also induce cell death. $p75^{NTR}$ serves as a proapoptotic receptor during developmental cell death and after injury to the nervous system (Fig. 1.7–1). Increases in $p75^{NTR}$ expression are responsible for apoptosis in embryonic retinas and sympathetic neurons during the period of naturally occurring neuronal death. Whereas BDNF binding to $p75^{NTR}$ in sympathetic neurons causes rapid cell death, NGF binding to the TrkA receptor on the same neurons provides a survival signal. In the context of neurotrophin processing, proneurotrophins are more effective than mature NGF in inducing $p75^{NTR}$-dependent apoptosis. These results suggest that the biological action of the neurotrophins can be regulated by proteolytic cleavage, with proforms preferentially activating $p75^{NTR}$ to mediate apoptosis and mature forms selectively activating Trk receptors to promote survival.

What are the reasons for having a neurotrophin receptor that mediates neuronal survival (Trk) and a receptor that mediates apoptosis ($p75^{NTR}$)? Neurotrophins may use a death receptor to prune neurons efficiently during periods of developmental cell death. In addition to competing for trophic support from the target, neurons must establish connections with the proper target. If neurons fail to establish connections with the proper target (also know as mistargeting), then they may undergo apoptosis. In this case, a neurotrophin may not only fail to activate Trk receptors but will bind to $p75^{NTR}$ and eliminate cells by an active killing process. For example, BDNF causes sympathetic cell death by binding to $p75^{NTR}$ when TrkB is absent. Likewise, NT-4 causes $p75^{NTR}$-mediated cell death in BDNF-dependent trigeminal neurons, due presumably to preferential $p75^{NTR}$ rather than TrkB stimulation. Therefore, Trk and $p75^{NTR}$ receptors can give opposite outcomes in the same cells. Cell death mediated by $p75^{NTR}$ may be important for the refinement of correct target innervations during development.

Retrograde Transport

Specificity of the biological effects of neurotrophins can also be modulated by the intracellular location of the neurotrophin ligand receptor complex. During development, neurotrophins are produced and released from the target tissues and become internalized into vesicles, which are then transported to the cell body. Interestingly, the biological effects of neurotrophins require that signals are conveyed over long distances from the nerve terminal to the cell body. Therefore, a central theme of the neurotrophic hypothesis is that neuronal survival and differentiation depend upon the retrograde signaling of trophic factors produced at the target tissue.

Each neurotrophin binds to transmembrane receptors and undergoes internalization and transport from axon terminals to neuronal cell bodies. Measurements of ^{125}I-NGF transport from distal axons to the cell body in compartment chambers indicate a rate from 3 to 10 mm per hour. Both Trk and $p75^{NTR}$ receptors undergo retrograde transport. The term "signaling endosome" has been coined to describe membrane vesicles that carry Trk, $p75^{NTR}$, and NGF.

A complex of NGF–TrkA has been found in clathrin-coated vesicles and endosomes, giving rise to the model that NGF and Trk are components of the retrograde signal. Several tyrosine-phosphorylated proteins are associated with the TrkA receptor during transport, suggesting that signaling by neurotrophins persists following internalization of their receptors. Internalization of NGF from axon terminals is necessary for phosphorylation and activation of the CREB transcription factor, which leads to changes in gene expression and increased neuronal cell survival. These events likely require the internalization and transport of activated Trk receptors and result in a survival response.

Neurotrophins and Synaptic Plasticity

Recent studies have established that neurotrophic factors play significant roles in influencing synaptic plasticity in the adult brain. Many neuronal populations are not only dependent upon these neurotrophins for their survival but also for modulating neuronal activity. Developmental regulation of synaptic plasticity in the visual system is illustrated by the formation of ocular dominance columns in layer 4 of the cortex, which can be strongly influenced by exogenous neurotrophins such as BDNF. Also, the effects upon the visual system can be observed using blocking antibodies for the neurotrophins as well neurotrophin antagonists (TrkB–IgG fusion proteins that bind neurotrophins), indicating that an alteration in the levels of endogenous neurotrophins has dramatic consequences.

Modulation of synaptic plasticity in the differentiated adult brain has also been demonstrated in the hippocampus in a series of studies. BDNF promoted the induction of a synaptic strengthening, termed long term potentiation (LTP), in hippocampal slices, while blocking reagents such as the TrkB–IgG fusion protein interfered with the induction of LTP. In addition, hippocampal preparations containing little or no BDNF gave rise to the same reduction in LTP, suggesting that there was a minimal quantity of BDNF required for the modulation of LTP. Subsequent addition of extra BDNF or adenoviral expression of BDNF to these preparations from mutant mice restored LTP. Neurotrophins have also been shown to evoke other forms of synaptic transmission. Exogenous BDNF or NT-3 has been shown to induce enhanced evoked responses in both hippocampal preparations

as well as neuromuscular junctions. Thus, neurotrophins can modulate synaptic strengthening and neurotransmission as well as promote cell survival and axonal and dendritic growth.

Neurotrophins and Behavior

A recent series of studies on genetically modified mice with reduced levels of BDNF have indicated striking effects upon adult brain function and behavior. These studies are important as earlier neurotrophin knockout mice studies were limited due to embryonic lethality or early postnatal death. However, heterozygous $BDNF^{+/-}$ mice in which BDNF levels are reduced by approximately one-half are viable and display a number of behaviors suggestive of impulse control abnormalities. In the absence of normal levels of BDNF, mice exhibit enhanced aggressiveness, hyperactivity, and hyperphagia. Intracerebroventricular infusion of BDNF or NT-4 led to a striking reversal of the feeding phenotype. In these heterozygous $BDNF^{+/-}$ mice, serotonergic neuronal functioning was abnormal in the forebrain, cortex, hippocampus, and hypothalamus. Most strikingly, administration of fluoxetine, a selective serotonin reuptake inhibitor, ameliorated the aggressive behavior, hyperphagia, ~~and hyperlocomotor activity.~~ In addition, a region-specific conditional deletion of BDNF in the brains of postnatal mice also led to hyperphagia, hyperactivity, as well as higher levels of anxiety as measured by a light/dark exploration test. This study and other conditional BDNF mice demonstrated that the feeding phenotype and the other behavioral abnormalities were mediated by the functioning of BDNF in the CNS as compared to any peripheral actions of the neurotrophin.

Lack of BDNF also created defects in memory tasks, consistent with defects in LTP found in the hippocampal slice studies. Heterozygous $BDNF^{+/-}$ mice had impairments in spatial memory tasks such as the Morris water maze. Abnormal behaviors elicited by partial deletion of BDNF indicate a significant role for this neurotrophin in higher-order behaviors, which have clinical correlates to psychiatric disorders, especially those associated with alteration in central serotonergic functioning.

OTHER NEUROTROPHIC FACTORS

Several prominent neurotrophic factor families carry out similar functions as the neurotrophins. Glial-derived neurotrophic factor (GDNF) is an 18-kDa protein, originally isolated from an astrocyte cell line and later shown to be made by many types of neurons. It represents one of the most potent trophic factors for dopaminergic neurons. In both in vitro and in vivo studies, GDNF has been shown to maintain the survival of dopaminergic neurons in the midbrain as well as neurons in the myenteric plexus in the gut. Due to its trophic effects on dopaminergic neurons it has been considered a potential therapeutic agent for Parkinson's disease.

GDNF binds to a protein, GFRα1, which is anchored to the plasma membrane by a glycophospholipid. Other ligands have also been discovered, namely, artemin, neurturin, and persephin, which recognize specific GFRα receptors. This ligand–receptor complex then associates with Ret, a receptor tyrosine kinase, which, like the Trk receptors, undergoes dimerization and becomes catalytically active. Phosphotyrosine-binding adaptor proteins such as Shc then bind to the Ret receptor and mediate downstream signaling cascades such as the MAP kinase pathway. Mutations in the Ret receptor and GFRα1 have been associated with Hirschprung's disease, a disorder caused by the lack of development of myenteric plexus neurons, leading to abnormal gut motility.

Ciliary neurotrophic factor (CNTF) belongs to a family of cytokines, including leukemia inhibitory factor (LIF) and interleukin-6, which maintain the survival of ciliary neurons as well as motor neurons. Due to its ability to rescue motor neurons after axotomy in animal studies, CNTF has been investigated as a therapeutic agent for motor neuron diseases such as amyotrophic lateral sclerosis (ALS). These factors utilize a receptor complex consisting of a plasma-membrane-bound CNTF-binding protein (CNTFα), a glycoprotein (gp130), and a LIF receptor (LIFR) to transduce signals. Upon formation of this complex, a soluble tyrosine kinase, the Janus kinase (JAK), is activated and leads to the activation of a specific family of transcription factors termed STATs.

Therefore, trophic factors exemplified by NGF, CNTF, and GDNF and their family members all utilize intracellular tyrosine phosphorylation to mediate neuronal cell survival. CNTF acts through a complex of a CNTF receptor, gp130, and LIFR subunits that are linked to the JAK/STAT signaling molecules, whereas the GDNF receptor consists of the c-Ret receptor tyrosine kinase and a separate α-binding protein.

CLINICAL CORRELATES

Neurotrophic factors regulate numerous neuronal functions in development and adult life and in response to injury. As a result, neurotrophins have been implicated in the pathophysiology of a wide variety of neurodegenerative and psychiatric disorders and have been considered as a therapeutic strategy for many neuropsychiatric disorders. It should be emphasized though that few human diseases affecting the nervous system have been shown to be caused by a defect in the neurotrophins or their receptors. Still, the finding that neurotrophic factors modulate neuronal survival and axonal growth was the initial rationale for potential clinical correlates to neurodegenerative disorders and neuronal injury such Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS as well as spinal cord injury. The additional effects of neurotrophic factors on synaptic connections, synaptic plasticity, and neurotransmission have formed the basis for their association with psychiatric disorders such as depression and substance abuse. In these conditions, the response to acute and chronic environmental changes leads to alterations in neuronal function.

The hypothesis underlying these clinical correlations as well as development of therapeutic strategies using neurotrophic factors assumes that these disease states result in either (1) decreased availability of neurotrophins for the affected neurons, (2) a decreased number of neurotrophin receptors on the affected neurons, and/or (3) decreased neuronal survival. These deficits can be ameliorated by the addition of neurotrophic factors. In all these disease states the assumption has been that exogenous neurotrophic factors would provide symptomatic treatment for the disease state rather than a cure for the core pathophysiology of these nervous system disorders.

Neurodegenerative Disorders

The initial clinical correlation to Alzheimer's disease was made in the 1980s based on studies on aged animals that showed that cholinergic neurons in the basal forebrain could be rescued with intracerebroventricular NGF, resulting in concomitant improvements in memory function. Subsequent animal studies of impaired motor neuron populations demonstrated that other neurotrophins, BDNF, NT-3, NT-4, and CNTF could rescue those neurons in an axotomized facial nerve and sciatic nerve. In addition, mutant mouse models of motor neuron disease (progressive motor neuron disease, *wobbler*), in which there was motor neuron degeneration, demonstrated that

BDNF and CNTF could increase the number of motor neurons and improve motor performance. These studies led to the therapeutic strategy to attempt to treat degenerative diseases affecting motor neurons with neurotrophins.

In the 1990s, great effort was focused on studying whether neurotrophic factors could be used as a treatment strategy for ALS, a progressive neurodegenerative disorder that specifically affects motor neurons and leads to death due to respiratory failure. With the development of recombinant forms of the neurotrophic factors, namely, BDNF, clinical trials have taken place on patients with ALS. Subcutaneous or intrathecal delivered BDNF had minimal beneficial effect and was associated with side effects such as pain and gastrointestinal symptoms. It was due to these side effects that decreased doses were used as compared to the doses in the animal studies. Similarly, use of another neurotrophic factor, CNTF, also led to even more significant side effects such as fever, pain, and anorexia, which also limited the doses used. These multisite clinical trials highlighted the challenges of delivery of large quantities of these proteins to CNS and PNS neurons. Similar clinical studies using NGF for the treatment of patients with Alzheimer's disease and diabetic neuropathy encountered similar hurdles involving problems of delivery and uncertain pharmacokinetics of the proteins.

Although these clinical trials have been disappointing, there is growing evidence that several specific neurodegenerative diseases would benefit from increasing the levels of neurotrophins. Huntington's disease (HD) is caused by a polyQ expansion in the ~~huntington~~ protein, which results in abnormal motor movements, personality changes, cognitive decline, and early death. Many studies have indicated that BDNF is a major target of mutant ~~huntington~~ protein. Decreased BDNF levels in the striatum have been detected in human HD subjects and mouse models of HD. A transgenic animal model in which BDNF has been specifically reduced in the cortex resulted in early dendritic changes, later loss of striatal medium ~~spinal~~ neurons, and early onset of clasping behavior. Moreover, gene expression profiling indicates that the depletion of BDNF in the cortex most closely resembles early grade human HD. These results suggest that striatal-specific atrophy in HD may be a consequence of a decrease of cortical BDNF by mutant ~~huntington~~.

Correlates to Psychiatric Disorders

Many functions of the neurotrophic factors in the adult CNS have been elucidated beyond their effects on survival. These functions include the maintenance of differentiated neuronal phenotypes and the regulation of synaptic connections, activity dependent synaptic plasticity, and neurotransmission. These additional functions have made neurotrophins attractive molecular intermediates that may be involved in the pathophysiology of psychiatric disorders in which environmental inputs can presumably lead to alterations in neuronal circuitry and ultimately behavior. In particular, it has become clear that neurotrophins can produce long-term changes by regulating transcriptional programs on the functioning of adult neurons. This could explain the long delay in therapeutic action of many psychiatric treatments. Again the clinical correlation is based on the assumption that there is a deficit in access or responsiveness to neurotrophic factors contributing to the phenotype of the disease state.

Major Depressive Disorder

The strongest evidence for a role for neurotrophins has come from the pathophysiology of depression, especially those associated with stress. For depression, it is believed that there is a fundamental dysregulation of synaptic plasticity and neuronal survival in regions of the brain such as the hippocampus. There are several lines of evidence suggesting a role of neurotrophins in depression. First, in animal models, restraint stress leads to decreased expression of BDNF in the hippocampus. In addition, chronic physical or psychosocial stress leads to atrophy and death of hippocampal neurons especially in the CA3 region in rodents and primates. Also, magnetic resonance imaging (MRI) studies have shown that patients with depressive or posttraumatic stress disorders exhibit a small decrease in hippocampal volume. It is unclear though whether the atrophy and/or death of these neurons is directly related to the decreased availability of BDNF. In addition, not all forms of depression are associated with stress. However, if structural remodeling and synaptic plasticity are involved in the cellular pathophysiology of depression, then BDNF is an attractive candidate molecule to mediate these alterations.

Exogenously administered BDNF in the hippocampus had antidepressant effects in two animal models of depression (i.e., the forced swim and learned helplessness paradigms) comparable to those of chronic treatment with pharmacological antidepressants. In addition, BDNF has also been shown to have trophic effects on serotonergic and noradrenergic neurons in vitro and in vivo. Mutant mice with decreased levels of BDNF have been shown to have a selective decrement in serotonergic neuron function and corresponding behavioral dysfunction consistent with serotonergic abnormalities.

Third, serotonin and norepinephrine reuptake inhibitor antidepressants upregulate CREB, a cyclic adenosine monophosphate (cAMP)-dependent transcription factor, and BDNF in a time course that corresponds to therapeutic action (10 to 20 days). The CREB transcription factor is involved in the induction of BDNF gene expression in neurons. This effect on the cAMP pathway provides a link between monoamine antidepressants and neurotrophin actions. These antidepressant treatments also lead to increases in expression of TrkB receptors in the hippocampus in a time course that also parallels the long time course of therapeutic action of these treatments. The effect of prolonged serotonin and norepinephrine reuptake inhibitor treatment involves enhancing neurotrophin signaling. Two other antidepressant treatments, monoamine oxidase inhibitors (MAOIs) and electroconvulsive therapy (ECT), also upregulate BDNF transcription. In rodents, long-term ECT has been shown to elicit the sprouting of hippocampal neurons that was attenuated in mutant mice that express lower levels of BDNF.

Conversely, exogenously administered BDNF in the mesolimbic dopamine system appears to have an opposite effect—increasing depressionlike behavior. In addition, removal of BDNF in this dopamine circuit appears to have antidepressant effects on a social defeat paradigm. These findings emphasize the complexity of BDNF's role in mediating aspects of behavior related to depression. Together, these studies provide a framework to examine further the neurotrophin system as a potential therapeutic target for the treatment of depression.

NEUROTROPHINS AND GENETICS

Until recently, no genetic association has been found between any neurotrophin and a human neurological or psychiatric disorder. A recent series of studies has linked one polymorphism in the BDNF gene with depression, bipolar disorder, and schizophrenia. This polymorphism identified from a single nucleotide polymorphism (SNP) screen leads to a single amino acid change from valine (Val) to methionine (Met) at position 66 in the pro region of the BDNF protein. This region is believed to be important in proper folding and intracellular sorting of the BDNF. Interestingly, proforms of neurotrophins have recently been shown to act as selective ligands for the p75 neurotrophin

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receptor. The mechanisms that contribute to altered $BDNF_{Met}$ function have been studied in neuronal culture systems. The distribution of $BDNF_{Met}$ to neuronal dendrites and its activity-dependent secretion are decreased. These trafficking abnormalities are likely to reflect impaired binding of $BDNF_{Met}$ to a sorting protein, sortilin, which interacts with BDNF in the prodomain region that encompasses the Met substitution.

This polymorphism is common in human populations with an allele frequency of 20 to 30 percent in Caucasian populations. This alteration in a neurotrophin gene correlates with reproducible alterations in human carriers. Humans heterozygous for the Met allele have smaller hippocampal volumes and perform poorly on hippocampal-dependent memory tasks. Using batteries of neuropsychological tests, carriers of the Met allele ~~(performed~~ worse on tasks that involved recalling places and events but did not differ from Val/Val individuals on tasks that have been classically shown to be less hippocampal-dependent, such as word learning and planning tasks.

However, genetic association studies for psychiatric disorders have presented a more complex picture. In patients with bipolar disorder, the Val allele appears to confer greater risk for the disease, while in patients with schizophrenia, depression, and anxiety disorders, there is little consensus as to whether the allele confers altered susceptibility. Inconsistency across genetic studies may be attributable to sampling and measurement issues, genetic heterogeneity due to differential sampling of populations, or a low frequency of homozygous Met carriers, which may lessen the effect size of any particular association. It may also relate to a failure to take into account relevant gene-by-gene and gene-by-environment interactions. This point is highlighted by a recent study of BDNF "knock-in" in mice ($BDNF^{Met/Met}$). The knock-in mice reproduced the phenotypic hallmarks related to hippocampal function that are seen in humans with this BDNF SNP. Subsequent analyses of these mice elucidated a phenotype that had not been established in human carriers: ~~Increased~~ anxiety. When stressed, $BDNF^{Met/Met}$ mice display increased anxiety-related behaviors, suggesting that environmental factors are likely required to elicit symptoms related to psychiatric disorders.

THERAPEUTIC POTENTIAL OF NEUROTROPHINS

The recent clinical trials have provided limits in designing therapeutic strategies to use neurotrophic factors for neurodegerative and psychiatric disorders. First, it has become clear that the physical delivery of sufficient quantities to target neurons is a major obstacle. Development of small molecules that readily cross the blood–brain barrier to activate neurotrophin receptors or potentiate the actions of neurotrophins is an approach that is in its infancy.

Second, because neurotrophins have multiple effects on neuronal activity, indiscriminate "flooding" of the CNS with neurotrophic factors will likely lead to untoward side effects such as epileptic activity. In addition, it had been noted in the clinical trials with BDNF that downregulation of the TrkB receptors after unregulated application of BDNF may have also contributed to the minimal therapeutic effects. New strategies are being studied that include more local and regulated application of neurotrophins through stereotactic injection of regulatable viral vectors or engineered progenitor cells. In particular, this approach is currently being applied to diseases such as Alzheimer's disease where there is a defined neuronal population such as basal forebrain cholinergic neurons that undergoes degeneration and is dependent on one neurotrophin such as NGF.

The activation of the neurotrophin system through other receptor signaling systems offers an alternative strategy. For example, antidepressant agents acting via monoamine G-protein-coupled receptors can lead to increased expression of both neurotrophins and neurotrophin receptors. Importantly, only the neurons that express the monoamine G-protein-coupled receptors will have enhanced production of the neurotrophin or Trk receptor. Recently, it has also been shown that other G-protein-coupled receptors, the purine adenosine 2A receptor, and pituitary adenylate-cyclase-activating peptide (PACAP) neuropeptide receptor can transactivate Trk neurotrophin receptors in the absence of neurotrophins in hippocampal neurons in vitro. Therefore, small molecules can activate Trk receptors in the absence of neurotrophins. These results raise the possibility that small molecules may be used to elicit neurotrophic effects for the treatment of neurodegenerative diseases by selective targeting of neurons that express specific G-protein-coupled receptors and Trk receptors.

It should be emphasized that the many possible treatment strategies that utilize neurotrophic factors are based on an assumption of symptomatic treatment of impaired neurons. This impairment implies not only cell survival but also proper functioning of these neurons. With greater understanding of the signal transduction pathways that are activated by neurotrophins, alternate strategies can be devised to manipulate these pathways through new drug development. In addition, further understanding of the core pathophysiological mechanism for neurodegenerative and psychiatric disorders will facilitate the development of rational therapies that involve engaging the neurotrophin system.

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▲ 1.8 Novel Neurotransmitters

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Neurotransmitters are chemicals that amplify or inhibit the depolarization signal from one neuron to that of an adjacent neuron. A neurotransmitter is typically released from a *presynaptic neuron* and travels across a small space, the *synaptic cleft* or *synapse*, to act upon the *postsynaptic neuron*. An action potential travels down a neuronal axon to the *presynaptic terminal*, a specialized appendage where neurotransmitters are stored in specialized *vesicles*. The action potential opens voltage-sensitive calcium channels in the membrane, allowing for an increase in cellular calcium that results in the vesicles releasing their contents into the synaptic cleft and acting upon receptors on the postsynaptic neuron membrane.

The definition of what a neurotransmitter is and is not has changed over the decades. The neurotransmitters initially discovered were small chemicals, first acetylcholine and later the biogenic amines such as serotonin, dopamine, norepinephrine, epinephrine, and histamine. Later it was found that amino acids and peptides could act as neurotransmitters, such as the case of enkephalin being the transmitter acting upon the opiate receptor. By the 1990s it became apparent that the neurotransmitter acting on cannabinoid receptors was derived from cellular lipids. Furthermore, even a gas, nitric oxide, could be a neurotransmitter, bypassing the requirement for postsynaptic receptors and acting directly within postsynaptic neurons. Figure 1.8–1 gives a visual for understanding the different types of agonists.

Fig. 1.8-1

GASES AS NEUROTRANSMITTERS

Nitric Oxide

The discovery that gases could function as neurotransmitters revealed that highly atypical modes of signaling existed between neurons. In the early 1990s, nitric oxide was the first gas to be ascribed a neurotransmitter function and proved to be an atypical neurotransmitter for several reasons. First, it was not stored in or released from synaptic vesicles, as it was a small gas it could freely diffuse into the target neuron. Second, its target was not a specific receptor on the surface of a target neuron, but intracellular proteins whose activity could directly be modulated by nitric oxide, leading to neurotransmission. Nitric oxide also lacks a reuptake mechanism to remove it from the synapse. Although enzymatic inactivation of it is postulated to exist, nitric oxide appears to have a very short half-life of a few seconds.

Nitric oxide was initially discovered as a bactericidal compound released from macrophages, and as an endothelial cell it derived relaxation factor allowing for the dilation of blood vessels. A role for nitric oxide in the brain followed, revealing a role for the gas in neurotransmission, learning and memory processes, neurogenesis, and neurodegenerative disease.

Synthesis of Nitric Oxide. Nitric oxide is chemically designated $NO^{\cdot}$, with the dot representing that the molecule is a free radical, also imparting a highly reactive nature. Nitric oxide is occasionally confused with nitrous oxide (N_2O), the gaseous anesthetic, and nitrogen dioxide (NO_2), a pollutant found in exhaust fumes, although these are not synthesized endogenously in mammals. However, a specific enzyme exists to generate nitric oxide within cells, nitric oxide synthase (Fig. 1.8–2). This enzyme generates nitric oxide by abstracting nitrogen from the amino acid, arginine, and reacting it with an oxygen atom. The enzyme utilizes nicotinamide adenine dinucleotide phosphate (NADPH) and generates citrulline as a byproduct.

Fig. 1.8-2

Three distinct enzymatic forms of nitric oxide synthase exist, each with differing locations and activation patterns within the body. *Neuronal nitric oxide synthase* (nNOS) was the first form discovered, by Bredt and Snyder, and is the predominant form in brain. nNOS is expressed only in neurons, especially those of the cortex, dentate gyrus of the hippocampus, corpus striatum, and cerebellum. Although nNOS containing neurons comprise only 1 percent of cortical neurons, their neuronal processes are so extensively distributed that almost all neurons make contact with an nNOS containing nerve terminus. nNOS enzyme activity is markedly augmented by calcium levels via the accessory protein calmodulin. Thus, nitric oxide may be synthesized following neuronal depolarization, in which calcium levels transiently increase.

Endothelial NOS (eNOS) is predominantly found in blood vessels, where it plays a profound role in allowing for the relaxation and dilation of blood vessels. Nitroglycerine and sodium nitroprusside exert their vasodilatory effects via conversion to nitric oxide. eNOS activity is augmented by phosphorylation and increases in intracellular calcium.

Inducible NOS (iNOS) exists in many tissues in miniscule amounts. However, its levels are strongly increased by a great variety of cell stressors, especially inflammation. In the brain it is largely induced in glial cells, but also in neurons.

Mechanism of Action of Nitric Oxide: Cyclic. Guanosine Monophosphate Long-term changes in brain function, such as learning and memory, involve a great variety of cellular processes,