NEUROTROPHIC FACTORS AND INTRACELLULAR SIGNAL TRANSDUCTION PATHWAYS

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A clear limitation of treating diseases of the central nervous system arises from the loss of regenerative potential of the brain at a very early age. Improper development of neural circuits or injury of neurons appears to be permanently fixed in the adult, with seemingly little hope of restorative therapy. This is most clearly true of the spectrum of neurologic diseases, such as neurodegenerative diseases (e.g., Alzheimer's or Parkinson's disease and stroke) and inflammatory disease (e.g., multiple sclerosis). In addition, breakthrough disorders such as epilepsy or myoclonus reflect inappropriate ''wiring'' or lack of appropriate feedback control of neuronal activity. It has also become apparent that some psychiatric diseases represent similar fixed deficits or lack of appropriate functional adaptation. Neurochemical and neuroanatomic deficits have been documented in affective diseases, schizophrenia, and anxiety disorders. Methods to recreate the early restorative potential of the brain would have great potential for significantly, and possibly permanently, reversing the often debilitating features of these neurologic and psychiatric diseases.

Over the last several years, of body of literature has delineated the important roles of neurotrophic factors in guiding the development of the nervous system. The first identified neurotrophic factor, nerve growth factor (NGF), was identified and characterized in the pivotal studies of Levi-Montalcini (1). The general properties of NGF now essentially define what neuroscientists consider a *neurotrophic factor.* A neurotrophic factor is capable of supporting the survival of at least one population of neurons in culture. It is secreted by a target tissue (either neuronal or nonneuronal) and acts on the neurons that innervate that tissue to support their survival or differentiation. Finally, a neurotrophic factor is expressed in the appropriate region and at the appropriate time in development to support the survival of a particular neuronal population. Although several variations and extensions of these principles have been delineated, the basics of defining a neurotrophic factor remain the same.

An exciting development in the neurosciences has been the realization that neurotrophic factors play important roles in the adult brain. The time course of neurotrophic factor expression is intriguing and indicates important function in the adult nervous system, as well as during development (2–4). The expression of these factors usually is very high during early development, a time of substantial growth, differentiation, and modeling of the nervous system. Later the levels generally drop, but they do not subside completely. In fact, in most cases in which it has been explored, the continued presence of these factors is substantial and is critical throughout adulthood (e.g., see refs. 5–7). Neuronal populations continue to depend on these factors for survival and optimal functioning.

More intriguing, although perhaps not surprising, studies have clearly shown that after development, these factors participate in the ongoing remodeling of neuronal function that underlies the adaptability or plasticity of neurons. In some cases, specific neurotrophic factors have been found to be necessary and sufficient for these changes to occur, from hippocampal plasticity and long-term potentiation (8–11) to the acquisition of new songs by songbirds (12–14). Models of neuronal now often incorporate components of neurotrophic factor signaling to explain synaptic alterations or strengthening. Contrary to the original models, these signaling events have been found not only to be retrograde signals from target neurons or other tissues, but also to be anterograde or autocrine signals. Furthermore, numerous studies have demonstrated that the expression of at least some of these factors can be rapidly regulated in the adult, a finding supporting a dynamic role in mediating responses to the environment.

Ongoing work in the neurotrophic factor field has been

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devoted to characterizing the pathways that underlie the intracellular signaling of these factors. This information may then be used to treat many different neurologic and psychiatric disorders, given the apparent critical roles of neurotrophic factors in the normal functioning and adaptability of the brain, as well as their potential to recapitulate early developmental processes to restore damaged or maladaptive neural systems. This review focuses on the neurobiology of these factors, their interactions with classical neurotransmitter systems, and their potential roles in the origin and possible treatment of psychiatric illnesses.

NEUROTROPHINS

The number of neurotrophic factors has now been expanded in to the dozens, each with unique a specificity in terms of biological activity, regional and temporal expression, and target specificity. Some factors previously known for their effects in other systems, such as insulin-like growth factors or tumor-derived factors, have now clearly been found also to be neurotrophic factors. These have been grouped into different families largely based on homology at the level of nucleotide sequence and therefore evolutionary relatedness. Perhaps the best understood and most widely expressed in the brain of these families are the *neurotrophins* (NTs) (2, 15). NGF is the prototype of the NT family, which also now includes brain-derived neurotrophic factor (BDNF), NT-3, and NT-4 (or NT-4/5). NGF has the most restricted specificity among these. NGF in the brain acts specifically on cholinergic neurons. In the rest of the nervous system, it also acts on sympathetic and sensory neurons. BDNF and NT-3 are widely and highly expressed, particularly in cortical and neocortical structures. NT-4 is also widely expressed, although generally at lower levels in the adult than are the others. The NTs are small, secreted proteins of about 12 kd that contain characteristic intramolecular disulfide bonds. These then form active noncovalent homodimers. They are found stored in vesicles clustered near the membrane. Each has been cloned and expressed in active recombinant forms.

TRK RECEPTORS

Generally better conserved than their ligands, the neurotrophic factor receptors also form families of related proteins (16,17). These receptors can be found in many different forms, from single, active proteins to large heteromeric complexes. Common to these are an extracellular ligand-binding portion, a mechanism to transduce this signal across the membrane, and at least one intracellular signaling apparatus. These may be contained in single proteins or distributed among several interacting proteins. Most, if not all, of the neurotrophic factor receptor complexes include a protein

tyrosine kinase. Although phosphorylation on tyrosine residues represents a relatively small proportion of all protein phosphorylation in the cell, it seems to be a critical part of neurotrophic factor signal transduction and function.

The NTs act through receptors known as *Trk receptors.* Given their name from a troponin/receptor kinase gene fusion identified from colon carcinoma, it has now been found that in their normal (protooncogene) forms, each Trk receptor contains a ligand-binding domain, a single transmembrane domain, and an intrinsic, intracellular tyrosine kinase domain (16,17). Each receptor, when transfected into a cell line, is capable of transducing the appropriate NT signals independently of other receptor proteins (18). There is specificity among the Trk receptors at physiologic NT concentrations. TrkA is a receptor for NGF, whereas TrkC is preferentially bound by NT-3. TrkB serves as a receptor for both BDNF and NT-4. The expression patterns of these receptors correlate with known sensitivity of those neurons to specific NTs. Several studies, particularly in mice with engineered deletions of NTs or their receptors, have shown significant complexity to these interactions. A review of this work falls beyond the scope of this review.

TRK DOCKING PROTEINS

On binding of an NT, the Trk receptor tyrosine kinase becomes activated. The most critical substrate of this activity appears to be the receptor itself. The receptor becomes rapidly autophosphorylated, and this is critical to receptor function. The receptor autophosphorylation sites form *docking sites* for the interaction of downstream signaling molecules (Fig. 16.1). Many signaling proteins contain domains that specifically bind to tyrosine residues when they are phosphorylated. Further binding specificity is mediated by the amino acids surrounding the autophosphorylated tyrosine. The domains of the signaling molecules that are used to bind the tyrosine residues seem to fall into a small number of conserved motifs (19).

Most proteins that bind to phosphorylated tyrosines fall into one of two groups. The most common phosphotyrosine binding motif is the *src-homology domain 2, or SH-2 domain.* SH-2 domains are typically identified based on their homology to other SH-2 domain–containing proteins. Some of these have been shown directly to possess specificity for phosphorylated tyrosines in the appropriate amino acid context. The SH-2 domain–containing proteins also often contain, or interact with proteins containing, an *src-homology domain 3, or SH-3* (20). The other, unrelated conserved motif that directs a separate type of specific protein–protein interaction has been termed, appropriately, a *phosphotyrosine binding domain, or PTB domain.* Proteins containing a PTB domain bind to a distinct set of phosphorylated tyrosine residues from those with SH-2 domains (21).

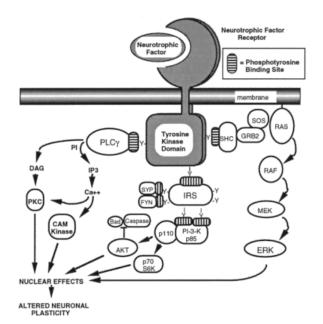After these signaling proteins bind to the activated, auto-

FIGURE 16.1. Neurotrophin intracellular signal transduction pathways. This schematic represents the three major signaling pathways emanating from a Trk-like tyrosine kinase receptor. The ligand, here a neurotrophin dimer, binds to its receptor and activates the tyrosine kinase activity. This results in autophosphorylation and phosphorylation of substrates. A complex forms in which docking proteins bind to the autophosphorylated receptor and are activated. These docking proteins bind to the receptor phosphorylation sites by SH2 domains (PLC-γ) or PTB domains (IRS and Shc). The three pathways shown here are as follows: (1) The PLC-γ pathway leads to the production of diacylglycerol and intracellular calcium. This results in activation of CAM kinases and PKC. (2) PI-3-Kinase (PI-3-K), comprising an 85-kd regulatory subunit and a 100-kd catalytic subunit, generally becomes activated by binding to an insulin receptor substrate (IRS)–like adaptor protein, which interacts with the receptor and then activates signaling proteins. IRS proteins also bind other signaling molecules such as Fyn and Syp. The PI-3-K then activates AKT and p70 S6-kinase (p70 S6K). AKT has an antiapoptotic effect through actions on Bad and caspaces. (3) Ras becomes activated through the stimulation of the GDP/GTP exchange activity of SOS, which is in a complex with Shc and Grb2. Activated Ras then turns on the cascade of kinases including Raf, MEK, and erks (also known as MAP kinases). Erks stimulate many known effectors. Each of these pathways then exerts a number of nuclear and nonnuclear actions with short- and long-term consequences for the cells.

phosphorylated receptor, they become activated. The mechanisms by which they are activated are not entirely clear. Often they, too, become phosphorylated on tyrosine residues. In addition, their recruitment to the membrane or into signaling complexes plays a role in the initiation of their activity (22,23). Activation of each of these NT-signaling proteins triggers distinct downstream cascades of target enzymes and other biological effects. Although there is great diversity of neurotrophic factor receptors, they seem to trigger only a few well-conserved types of downstream signaling pathways. Among the best characterized of the pathways include the Ras/extracellular signal regulated kinase (ERK) pathway, the phosphotidylinositol-3′-OH-kinase (PI-3-K)

pathway, and the phospholipase C-γ (PLC-γ) pathway (24). In addition, specific tyrosine phosphatases are activated that modulate these responses and may contain pathway-activating properties of their own.

NEUROTROPHIC FACTOR INTRACELLULAR SIGNALING PATHWAYS: RAS/ERK (MAPK) CASCADE

The Ras/ERK pathway is regulated by the activity of the Ras proteins. Ras is a small, membrane-associated protein that serves as a transducer of signal from tyrosine kinase activity to ERK proteins, among other activities (25). The activity of Ras depends on the type of the guanine nucleotide it is bound to. Hence, Ras is a G protein, although distinct from the heterotrimeric G proteins coupled to many neurotransmitter receptors. Ras is active when binding guanosine triphosphate (GTP), but at rest it is inactive and bound to guanosine diphosphate (GDP). Activation of Trk receptor tyrosine kinases leads to the binding of an adapter protein in the Shc family. Shc becomes tyrosine phosphorylated and binds a Grb protein, such as Grb2. Shc contains a PTB domain and an SH-2 domain, whereas Grb2 contains two SH-2 domains and a SH-3 domain. This complex then activates a GDP-GTP exchange factor, such as SOS, which, in turn, activates Ras through GTP binding.

Once activated, Ras recruits a serine kinase of the Raf family to the membrane, where it is activated. This initiates a cascade in which Raf activates MEK (from *M*APK or *E*RK *k*inase), and then MEK phosphorylates and activates ERKs (26). ERKs, also known as mitogen-activated protein kinase (MAPKs), are abundant, multifunctional, intracellular kinases with many different cellular activities. ERKs have been shown to phosphorylate such diverse proteins as tyrosine hydroxylase, transcription factors, regulators of protein translation, microtubule proteins, and many others (27,28). In cells *in vitro*, ERKs have been shown to mediate neuron survival, neuritic process elongation (29), and levels of specific neuronal enzymes and ion channels, among other effects (30). ERKs have been shown to be important in hippocampal long-term potentiation in brain slices (31). Some effects of ERK activation are very rapid, whereas others are delayed and persistent.

PLC-γ CASCADE

A second NT-signaling pathway involves PLC-γ activation (32,33). Like the better-understood PLC-β, PLC-γ cleaves phosphatidylinositol phosphates into diacylglycerol and inositol phosphates. Diacylglycerol can activate protein kinase C (PKC), whereas inositol-1,4,5-tris phosphate releases intracellular stores of calcium. Intracellular calcium can exert numerous effects from the activation of Ca^{2+}/calmo-

dulin–dependent protein kinases to the production of cyclic adenosine monophosphate through some adenylyl cyclases. All these are known to have powerful effects on neurons. Unlike PLC-β, which is regulated by heterotrimeric G-protein–coupled receptors, PLC-γ is regulated by tyrosine phosphorylation (34). PLC-γ contains SH-2 and SH-3 domains. When bound to tyrosine phosphorylated receptors, it is recruited to the membrane and becomes phosphorylated, which activates its PLC activity. Virtually nothing is known about the role of PLC-γ in the intact brain, although it is likely to exert important effects on neuronal function.

PI-3-K CASCADE

Somewhat less well understood is the PI-3-K pathway (35–37). Several types of PI-3-Ks have been identified. Type 1 PI-3-Ks are regulated by tyrosine kinase activity. They are heterodimers of a catalytic α subunit and a regulatory β subunit. The β subunit contains SH-2 and SH-3 domains. When bound to phosphorylated tyrosines, the β subunit activates the catalytic activity of the α subunit. This phosphorylates phosphatidylinositol on the 3′-hydroxl group (distinct from the 4′,5′ phosphorylated forms mentioned earlier). Furthermore, PI-3-K has been shown to possess protein kinase activity and can bind Ras (38). The PI-3-K lipid product, phosphotidylinositol-3′-phosphate, activates at least two protein kinases, AKT and S6-kinase. AKT is best known for its powerful ability to oppose programmed cell death (i.e., antiapoptotic effects), although it has other metabolic actions as well. S6-kinase is named for its ability to phosphorylate the ribosomal subunit S6 (although not to be confused with ribosomal S6-kinase RSK), and it has numerous other cellular effects as well. Currently, these effects are less well elucidated than those of the ERKs. Within neurons, PI-3-K has been shown to mediate cell survival, initiation of neuritic process outgrowth, and acquisition of sensitivity to glutamate excitotoxicity (39), among other actions. Again, little is known about its role in intact brain.

Although PI-3-K possesses some ability to bind phosphorylated receptors itself, it seems largely to be activated by receptors through docking proteins. Important PI-3-K docking proteins are the insulin receptor substrate (IRS) family of proteins (40). IRS1, IRS2, and IRS4 are expressed in brain (41). More distantly related PI-3-K docking proteins are the GAB family of proteins. All these bind to the receptors through PTB domains and become phosphorylated on numerous tyrosines. Most of these tyrosines are then bound by PI-3-K, leading to a substantial amplification of PI-3-K signaling. IRS proteins can be bound by other signaling molecules such as protein tyrosine phosphatases and also possess numerous serine and threonine phosphorylation sites. Therefore, it is likely that the IRS proteins are important sites of convergence of numerous types of signaling pathways.

INTERACTION OF NEUROTROPHIN SIGNALING CASCADES

Numerous levels of complexity have been found in the downstream signaling pathways of the NT receptors. PI-3-K has been shown to contribute to ERK activation by Ras-dependent and Ras-independent process pathways (36,38, 42). Ras can contribute to activation of AKT, and both SHC and IRS can bind to the same phosphorylated tyrosine site, although with differing affinities (43). PLC-γ can activate ERK in neurons and can theoretically terminate PI-3-K signaling by cleaving phosphotidylinositol-3′-phosphate. In addition, most of these proteins exist in multiple isoforms arising either from different genes or differential splicing of the same gene. These isoforms are differentially expressed during development and in different brain regions, although there is also a great deal of overlap. The complement of signaling proteins and adaptors would be expected to determine the effects of the NT on particular populations of neurons. Regulation of these proteins may influence plasticity or other neuronal responses. Furthermore, this complexity of expression and cross-talk allows tremendous opportunity for potential sites of therapeutic intervention.

REGULATION OF NEUROTROPHIN SIGNALING BY ACTIVATION OF G-PROTEIN–COUPLED RECEPTORS

There is also evidence of cross-talk between G-protein–coupled receptor signaling pathways and the NT cascades. Many different types of interactions occur between the G-protein receptor–coupled second messenger-dependent pathways and the Trk signaling pathways. Only a few are discussed here, to demonstrate the complexity and possible types of interactions.

Activation of Trk Docking Proteins and the Ras/ERK Pathway by G-Protein–Coupled Receptors

G-protein–coupled receptors are reported to activate the Ras/ERK by a pathway that is independent of their respective second messenger systems (44). This alternate pathway is dependent on internalization of the receptor and recruitment of a soluble tryosine kinase that directly phosphorylates the adaptor proteins (Shc and Gab) that lead to activation of Ras and subsequently the Ras/ERK pathway. For example, internalization of the β-adrenergic receptor (βAR) leads to binding of β-arrestin, which inhibits activation of the receptor. Studies demonstrated that β-arrestin also functions as an adaptor protein that binds both βAR and a soluble tyrosine kinase, Src. Studies demonstrated that 5-hydroxytryptamine (5-HT$_{1A}$) receptors activate the Ras/ERK pathway, possibly through this mechanism (45). Reg-

ulation of the Ras/ERK pathway by internalization of G-protein–coupled receptors is not observed in all cases (e.g., 5-HT$_{2A}$ receptor), a finding indicating receptor or cellular specificity in the control of this pathway.

Numerous other potential mechanisms of cross-talk between G protein and neurotrophic factor signaling pathways have been identified. For instance, several other forms of PI-3-Ks have been elucidated, some of which are activated by G-protein–coupled receptors, but presumably they lead to at least some similar downstream signaling effects through the production of similar phosphorylated inositol lipids (35–37). Similarly, PLC-β, the G-protein–coupled form of PLC, would be expected to produce at least some of the same effects as PLC-γ by activation of PKCs and mobilization of calcium. Furthermore, both PKC and levels of cyclic adenosine monophosphate modulate the activity of Raf and hence the ERK pathway (46–49). Calcium mobilization can activate ERKs through the intracellular tyrosine kinase, Pyk2 (50). Obviously, the potential for cross-talk with G-protein–coupled systems is great, and sorting out the mechanisms relevant in various brain regions and mechanisms of plasticity is an important area for investigation.

SOME OTHER CLASSES OF NEUROTROPHIC FACTORS AND THEIR SIGNALING SYSTEMS

Although this review focuses on the NT family of neurotrophic factors, several others have generated significant interest (Fig. 16.2). For example, the glial cell line–derived neurotrophic factor (GDNF) family of proteins has been found to have profound effects on dopaminergic and motor neurons, as well as enteric neurons (51–53). Thus far, this family includes GDNF, artemin, persephin, and neurturin. This family, distantly related to the transforming growth factor-β (TGF-β) family, signals through a heteromeric signaling complex (54). A common component of all the GDNF receptor complexes identified is the Ret protein. Ret spans the membrane and contains an intracellular tyrosine kinase domain. This domain, which is less well characterized than for the Trk receptor, can nonetheless signal at least some of the same intracellular signaling pathways, including the Ras/ERK pathway and PI-3-K (55). Ret associates with various receptor-binding proteins (GFR-α subunits 1 through 4). These subunits do not span the membrane, but are linked to the extracellular surface through a glycosyl-phosphatidylinositol moiety. The GFR-α subunits provide specificity for ligand binding and participate in the activation of the Ret tyrosine kinase. Furthermore, evidence indicates that they can stimulate activation of src-like tyrosine kinases independent of Ret. The known activities of the GDNF family of proteins has spurred interest in its role in the pathogenesis and possible treatment of diseases such
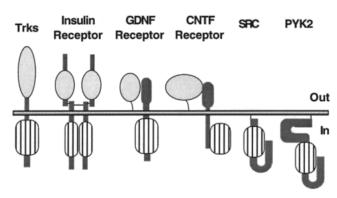

FIGURE 16.2. Other neurotrophic factor signaling cascades. This cartoon illustrates the general motifs used by neurotrophic factor receptors to transduce a signal across the membrane and to couple with the intracellular signaling pathways. The *light gray ovals* represent extracellular (Out) ligand-binding domains. The *vertical striped* intracellular (In) ovals are tyrosine kinase domains. The Trk receptors, like EGF receptors, are single polypeptide chains that span the membrane. These contain intrinsic extracellular ligand binding domains and intracellular tyrosine kinase activity. The insulin and IGF receptors are derived from the cleavage and subsequent disulfide linking of two identical chains. These receptors also contain intrinsic binding and kinase activities. The GDNF receptor is a heteromeric complex in which the ligand-binding specificity resides within a separate phosphatidylinositolglycan-linked subunit (GFR-α). The signal from the ligand–GFR-α complex is then transduced across the membrane by Ret, which contains an intracellular tyrosine kinase domain. The CNTF receptor is typical of the cytokine receptors, which contain separate binding and transmembrane subunits, and then couple to a soluble intracellular tyrosine kinase (a JAK). Other systems couple to the tyrosine kinase intracellular signaling pathway by interacting with one of several membrane-associated intracellular tyrosine kinases. Some G proteins, for instance, act through src-like proteins, whereas intracellular calcium can activate Pyk2, an intracellular tyrosine kinase in the focal adhesion kinase family.

as Parkinson's disease, addiction, and amyotrophic lateral sclerosis (52,53).

Another large class of receptors couples to an intracellular tyrosine kinase known as the Janus kinase, or JAK (56, 57). These receptor kinases include extracellular binding components, one of which spans the membrane but does not have intrinsic kinase activity. Instead, they bind to and activate specific members of the JAK family of kinases. The JAKs then activate certain intracellular effector molecules, including IRS proteins, SHC, and others. Moreover, they interact with a unique group of proteins called STATs. STAT proteins bind to JAK. After tyrosine phosphorylation, they are released and translocated to the nucleus, where they function directly as DNA-binding transcriptional activators. Many neurotrophic factors activate the JAK/STAT pathway, including ciliary neurotrophic factor, growth hormone, leptin, and many cytokines. Characterization of the role of STAT-mediated transcription in brain lags behind that of other earlier identified transcription factors, but it is an area of intensive study.

Even factors known for their hormone functions or pe-

ripheral effects have been found to have substantial activity in the central nervous system. Insulin and insulin-like growth factor 1 (IGF-1), and their respective transmembrane receptor tyrosine kinases, are expressed widely in brain and play roles in development and behavior (58,59). Furthermore, epidermal growth factor (EGF) and EGF-like ligands in the TGF-α family are expressed in brain along with their receptor, the EGF receptor. Evidence is accumulating for roles for these factors in the adult central nervous system as well (60). Although not discussed in detail, these serve as further examples of the complexity of neurotrophic factor signaling at many levels in the brain. These receptors also share coupling to the same modules of signaling pathway proteins as the Trks and Ret. Apparently, there is a tremendous diversity of neurotrophic ligands and ligand-binding domains within their receptors that allows fine anatomic and temporal specificity of action, along with the potential for synergistic or counterregulatory mechanisms. However, the relatively smaller number of conserved signaling pathways to which they couple suggests that they share common mechanisms of action to shape neuronal responses.

ROLE OF NEUROTROPHIC FACTORS IN THE ACTIONS OF PSYCHOTROPIC DRUGS

As investigations of the psychotropic drugs have been extended, it has become clear that these agents also influence the expression of neurotrophic factors and their signal transduction systems. Regulation of neurotrophic factor signaling could thereby contribute to the desired actions of therapeutic of agents, as well as the negative effects of other drugs. These possibilities are illustrated briefly in this section.

Regulation of Neurotrophin Systems by Stress and Antidepressant Treatment

Preclinical and clinical studies have reported an atrophy or loss of neurons in limbic brain structures that could be related to dysfunction of neurotrophic factor systems. Chronic physical or social stress can result in atrophy or death of stress-vulnerable neurons in the hippocampus of rodents and nonhuman primates (61,62). More recently, investigators conducting brain imaging studies reported that the volume of the hippocampus is reduced in patients suffering from depression of posttraumatic stress disorder (63). Postmortem studies also demonstrate that the numbers of neurons and glia in prefrontal cortex are reduced in patients with depression (63). The expression of BDNF in hippocampus is decreased by exposure of animals to stress (64). This effect could contribute to the atrophy and death of hippocampal neurons, although it is also likely that other pathways are involved in this effect (61,62).

In contrast to the actions of stress, antidepressant treatment increases the expression of BDNF, as well as its recep-

tor TrkB, in hippocampus (65,66). Up-regulation of BDNF is dependent on long-term antidepressant treatment, consistent with the time course for the therapeutic action of these agents. Both norepinephrine and serotonin-selective reuptake inhibitor antidepressants increase BDNF expression, a finding suggesting that this NT system may be a common postreceptor target of these monoamines and antidepressant treatment. In addition, nonantidepressant psychotropic drugs do not increase BDNF expression in hippocampus, a finding indicating that this effect is specific to antidepressants. The possibility that BDNF contributes to the therapeutic actions of antidepressants is supported by behavioral studies. Infusion of BDNF into midbrain or hippocampus produces antidepressant-like effects in behavioral models of depression, the forced swim test, and learned helplessness paradigms (67,68). Additional studies will be required to elucidate further the role of BDNF, as well as other neurotrophic factors, in the pathogenesis and treatment of depression. However, these findings have contributed to an exciting new hypothesis of depression.

Role of Neurotrophic Factors in the Actions of Drugs of Abuse

A picture is emerging that neurotrophic factors and their signaling pathways play important roles in mediating acute and chronic changes in synaptic connectivity, neuronal physiology, and gene expression. A powerful and important model of environmentally induced acute and persistent alterations in brain function is the effect of chronic exposure to drugs of abuse (69,70). Within laboratory animals, exposure to any of several diverse addicting drugs leads a set of alterations in neuronal biochemistry, electrophysiology, and morphology in specific brain regions implicated in addictive behaviors. Concomitantly, these animals display alterations in behavior including tolerance, dependence, sensitization, craving, and drug-seeking behaviors reminiscent of the behaviors seen in humans suffering from drug addiction. Specifically, alterations in the dopaminergic nucleus, the ventral tegmental area (VTA), are reminiscent of the changes seen with neurotrophic factor withdrawal in cell culture: the cells become smaller with less prominent neuritic processes, they have decreased neurofilament expression and axoplasmic transport, and they have decreased expression and accumulation of tyrosine hydroxylase, the rate-limiting enzyme in dopamine syntheses and a critical neuron type-specific protein. Infusing NTs such as BDNF or GDNF into the VTA can restore most, or all, of these features to normal (71,72). Additional studies have also implicated endogenous NT-3 in the drug-induced changes (73). Furthermore, the role of ERK signaling in this system has been established. ERK activity is increased in the VTA by chronic morphine exposure (74). Infusion of a specific antisense oligonucleotide against ERK1 into the VTA again blocks the morphine-induced biochemical changes (75).

Dissecting the mechanisms of signaling protein regulation within specific brain nuclei in the intact animal poses special challenges. However, using tools from *in vitro* studies, headway is now starting to be made. For instance, the mechanism of this ERK up-regulation is unclear, but it has been shown that PLC-γ, which is capable of activating ERK, is up-regulated in VTA after chronic morphine exposure (76). Although levels of the neurotrophic factors themselves have not been found to be significantly altered in VTA by chronic drug exposure, they may be regulated indirectly by modulation of their signaling systems.

CONCLUSIONS

The neurotrophic factors and their signal transduction cascades represent a complex array of pathways that influence many aspects of neuronal function and survival during development as well as in the adult central nervous system. The characterization of these pathways has provided many new target sites for the development of novel agents that could be used to treat a variety of neurologic and psychiatric illnesses. There is currently a tremendous amount of interest in this area, and agents are already available for selective blockade of certain components of the Ras/ERK pathway. Moreover, characterization of the roles these pathways play in the normal nervous system may lead to identification of abnormal conditions that underlie pathologic states. The opening of the field of growth factor action into the neurosciences opens opportunities that may be as rich or as powerful, if not more, as those that have been presented with the more traditional neurotransmitter systems.

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REFERENCES

1. Levi-Montalcini R. The nerve growth factor 35 years later. *Science* 1987;237:1154–1162.
2. Barde YA. The nerve growth factor family. *Prog Growth Fact Res* 1990;2:237–248.
3. Lu B, Figurov A. Role of neurotrophins in synapse development and plasticity. *Rev Neurosci* 1997;8:1–12.
4. Chao MV. Trophic factors: an evolutionary cul-de-sac or door into higher neuronal function? *J Neurosci Res* 2000;59:353-355.
5. Maisonpierre PC, Belluscio L, Friedman B, et al. NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron* 1990;5:501–509.
6. Friedman WJ, Ernfors P, Persson H. Transient and persistent expression of NT-3/HDNF mRNA in the rat brain during postnatal development. *J Neurosci* 1991;11:1577–1584.
7. Timmusk T, Belluardo N, Metsis M, et al. Widespread and developmentally regulated expression of neurotrophin-4 mRNA x in rat brain and peripheral tissues. *Eur J Neurosci* 1993;5:605–613.
8. Kang H, Schuman EM. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* 1995;267:1658–1662.
9. Korte M, Carroll P, Wolf E, et al. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci USA* 1995;92:8856–8860.
10. Patterson SL, Abel T, Deuel TA, et al. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* 1996;16:1137–1145.
11. Messaoudi E, Bårdsen K, Srebro B, et al. Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. *J Neurophysiol* 1998;79:496–499.
12. Akutagawa E, Konish M. Transient expression and transport of brain-derived neurotrophic factor in the male zebra finch's song system during vocal development. *Proc Natl Acad Sci USA* 1998;95:11429–11434.
13. Rasika S, Alvarez-Buylla A, Nottebohm F. BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. *Neuron* 1999;22:53–62.
14. Dittrich F, Feng Y, Metzdorf R, et al. Estrogen-inducible, sex-specific expression of brain-derived neurotrophic factor mRNA in a forebrain song control nucleus of the juvenile zebra finch. *Proc Natl Acad Sci USA* 1999;96:8241–8246.
15. Thoenen H. The changing scene of neurotrophic factors. *Trends Neurosci* 1991;14:165–170.
16. Barbacid M. The Trk family of neurotrophin receptors. *J Neurobiol* 1994;25:1386–1403.
17. Ip NY, Yancopoulos GD. Neurotrophic factors and their receptors. *Ann Neurol* 1994;35:S13–S16.
18. Ip NY, Stitt TN, Tapley P, et al. Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. *Neuron* 1993;10:137–149.
19. Ullrich A, Schlessinger J. Growth factor signaling by receptor tyrosine kinases. *Neuron* 1992;9:383–391.
20. Schlessinger J. SH2/SH3 signaling proteins. *Curr Opin Genet Dev* 1994;4:25–30.
21. van der Geer P, Pawson T. The PTB domain: a new protein module implicated in signal transduction. *Trends Biochem Sci* 1995;20:277–280.
22. Stokoe D, Macdonald SG, Cadwallader K, et al. Activation of Raf as a result of recruitment to the plasma membrane. *Science* 1994;264:1463–1467.
23. Leevers SJ, Paterson HF, Marshall CJ. Requirement for Ras in Raf activation is overcome by targeting Raf to the plasma membrane. *Nature* 1994;369:411–414.
24. Russell DS. Neurotrophins: mechanisms of action. *Neuroscientist* 1995;1:3–6.
25. Denhardt DT. Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. *Biochem J* 1996;318:729–747.

26. Ahn NG, Seger R, Krebs EG. The mitogen-activated protein kinase activator. *Curr Opin Cell Biol* 1992;2:992–999.

27. Davis RJ. The mitogen-activated protein kinase signal transduction pathway. *J Biol Chem* 1993;268:14553–14556.

28. Cobb MH. MAP kinase pathways. *Prog Biophys Mol Biol* 1999; 71:479–500.

29. Fukuda M, Gotoh Y, Tachibana T, et al. Induction of neurite outgrowth by MAP kinase in PC12 cells. *Oncogene* 1995;11: 239–244.

30. Grewal SS, York RD, Stork PJ. Extracellular-signal-regulated kinase signalling in neurons. *Curr Opin Neurobiol* 1999;9: 544–553.

31. English JD, Sweatt JD. A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *J Biol Chem* 1997;272:19103–19106.

32. Wahl MI, Jones GA, Nishibe S, et al. Growth factor stimulation of phospholipase C-gamma 1 activity: comparative properties of control and activated enzymes. *J Biol Chem* 1992;267: 10447–10456.

33. Carpenter G, Ji QS. Phospholipase C-gamma as a signal-transducing element. *Exp Cell Res* 1999;253:15–24.

34. Nishibe S, Wahl MI, Hernandez-Sotomayor SM, et al. Increase of the catalytic activity of phospholipase C-gamma 1 by tyrosine phosphorylation. *Science* 1990;250:1253–1256.

35. Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Annu Rev Biochem* 1998;67:481–507.

36. Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* 1998;1436:127–150.

37. Anderson RA, Boronenkov IV, Doughman SD, et al. Phosphatidylinositol phosphate kinases, a multifaceted family of signaling enzymes. *J Biol Chem* 1999;274:9907–9910.

38. Rodriguez-Viciana P, Warne PH, Dhand R, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 1994;370: 527–532.

39. Fryer HJ, Wolf DH, Knox RJ, et al. Brain-derived neurotrophic factor induces excitotoxic sensitivity in cultured embryonic rat spinal motor neurons through activation of the phosphatidylinositol 3-kinase pathway. *J Neurochem* 2000;74:582–595.

40. Myers MG Jr, White MF. Insulin signal transduction and the IRS proteins. *Annu Rev Pharmacol Toxicol* 1996;36:615–658.

41. Numan S, Russell DS. Discrete expression of insulin receptor substrate-4 mRNA in adult rat brain. *Brain Res Mol Brain Res* 1999;72:97–102.

42. Carpenter CL, Cantley LC. Phosphoinositide kinases. *Curr Opin Cell Biol* 1996;8:153–158.

43. Wolf G, Trub T, Ottinger E, et al. PTB domains of IRS-1 and Shc have distinct but overlapping binding specificities. *J Biol Chem* 1995;270:27407–27410.

44. Luttrell LM, Ferguson SSG, Daaka Y, et al. β-Arrestin-dependent formation of β₂ adrenergic receptor-Src protein kinase complexes. *Science* 1999;283:655–661.

45. Mendez J, Kadia TM, Somayazula RK, et al. Differential coupling of serotonin 5-HT1A and 5-HT1B receptors to activation of ERK2 and inhibition of adenylyl cyclase in transfected CHO cells. *J Neurochem* 1999;73:162–168.

46. Sozeri O, Vollmer K, Liyanage M, et al. Activation of the c-raf protein kinase by protein kinase C. *Oncogene* 1992;7: 2259–2262.

47. Kolch W, Heldecker G, Kochs G, et al. Protein kinase C activates RAF-1 by direct phosphorylation. *Nature* 1993;364:249–252.

48. Wu J, Dent P, Jelinek T, et al. Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. *Science* 1993;262:1065–1069.

49. Cook SJ, McCormick F. Inhibition by cAMP of ras-dependent activation by raf. *Science* 1993;262:1069–1072

50. Lev S, Moreno H, Martinez R, et al. Protein tyrosine kinase PYK2 involved in Ca(2 +)-induced regulation of ion channel and MAP kinase functions. *Nature* 1995;376:737–745.

51. Lin LF, Doherty DH, Lile JD, et al. GDNF: a glial cell line–derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 1993;260:1130.

52. Shen L, Figurov A, Lu B. Recent progress in studies of neurotrophic factors and their clinical implications. *J Mol Med* 1997; 75:637–644.

53. Gash DM, Zhang Z, Gerhardt G. Neuroprotective and neurorestorative properties of GDNF. *Ann Neurol* 1998;44:S121–S125.

54. Treanor JJ, Goodman L, de Sauvage F, et al. Characterization of a multicomponent receptor for GDNF. *Nature* 1996;382: 80–83.

55. van Weering DH, Bos JL. Signal transduction by the receptor tyrosine kinase Ret. *Recent Results Cancer Res* 1998;154:271–281.

56. Stahl N, Yancopoulos GD. The tripartite CNTF receptor complex: activation and signaling involves shared with other cytokines. *J Neurobiol* 1994;25:1454–1466.

57. Cattaneo E, Conti L, De-Fraja C. Signalling through the JAK-STAT pathway in the developing brain. *Trends Neurosci* 1999; 22:365–369.

58. Adamo M, Raizada MK, LeRoith D. Insulin and insulin-like growth factor receptors in the nervous system. *Mol Neurobiol* 1989;3:71–100.

59. Arsenijevic Y, Weiss S. Insulin-like growth factor-I is a differentiation factor for postmitotic CNS stem cell–derived neuronal precursors: distinct actions from those of brain-derived neurotrophic factor. *J Neurosci* 1998;18:2118–2128.

60. Yamada M, Ikeuchi T, Hatanaka H. The neurotrophic action and signalling of epidermal growth factor. *Prog Neurobiol* 1997; 51:19–37.

61. Sapolsky RM. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress* 1996;1:1–19.

62. McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci* 1999;22:105–122.

63. Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* 1999;46:1181–1191.

64. Smith MA, Makino S, Kvetnansky R, et al. Stress alters the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 1995;15: 1768–1777.

65. Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 1995;15:7539–7547.

66. Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 1996; 16:2365–2372.

67. Siucak JA, Lewis DR, Wiegaud SJ, et al. Antidepressant-like effect of brain derived neurotrophic factor (BDNF). *Pharmacol Biochem Beh*; 56:121–127.

68. Shirayama Y, Chen ACH, Duman RS. Antidepressant-like effects of BDNF and NT-3 in behavioral models of depression. *Soc Neurosci Abstr* 2000;387:2.

69. Nestler EJ. Molecular mechanisms of opiate and cocaine addiction. *Curr Opin Neurobiol* 1997;7:713–719.

70. Koob GF, Nestler EJ. The neurobiology of addiction. *J Neuropsychiatry Clin Neurosci* 1997;9:482–497.

71. Berhow MT, Russell DS, Terwilliger RZ, et al. Influence of neurotrophic factors on morphine- and cocaine-induced biochemical changes in the mesolimbic dopamine system. *Neuroscience* 1995; 68:969–979.

72. Messer CJ, Eisch AJ, Carlezon WA Jr, et al. Role for GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron* 2000;26:247–257.

73. Pierce RC, Pierce-Bancroft AF, Prasad BM. Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the ras/mitogen-activated protein kinase signal transduction cascade. *J Neurosci* 1999;19: 8685–8695.

74. Ortiz J, Harris HW, Guitart X, et al. Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. *J Neurosci* 1995;15:1285–1297.

75. Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci* 1996;16: 4707–4715.

76. Wolf DH, Numan S, Nestler EJ, et al. Regulation of phospholipase C-gamma in the mesolimbic dopamine system by chronic morphine administration. *J Neurochem* 1999;73:1520–1528.