CHAPTER 3

The Role of BDNF in Epilepsy and Other Diseases of the Mature Nervous System

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Abstract

The neurotrophin brain-derived neurotrophic factor (BDNF) is ubiquitous in the central nervous system (CNS) throughout life. In addition to trophic effects on target neurons, BDNF appears to be part of a general mechanism for activity-dependent modification of synapses in the developing and adult nervous system. Thus, diseases of abnormal trophic support (such as neurodegenerative diseases) and diseases of abnormal excitability (such as epilepsy and central pain sensitization) can be related in some cases to abnormal BDNF signaling. For example, various studies have shown that BDNF is upregulated in areas implicated in epileptogenesis, and interference with BDNF signal transduction inhibits the development of the epileptic state. Further study of the cellular and molecular mechanisms by which BDNF influences cell survival and excitability will likely provide novel concepts and targets for the treatment of diverse CNS diseases.

BDNF: Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the "neurotrophin" family of neurotrophic factors. It was originally purified from pig brain due to its survival-promoting action on a subpopulation of dorsal root ganglion neurons.[1] The amino acid sequence of BDNF has a strong homology with nerve growth factor (NGF), the neurotrophin (NT) first described due to its trophic (survival and growth-promoting) effects on sensory and sympathetic neurons. Since the discovery of NGF in the early 1950s by Rita Levi-Montalcini and Viktor Hamburger[2] and the discovery of BDNF by Yves Barde and colleagues in 1982,[1] other members of the NT family such as neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) have been described. Each NT appears to have a unique profile of trophic effects on distinct subpopulations of peripheral nervous system and central nervous system neurons.

BDNF Structure

The mature form of human BDNF has been mapped to chromosome 11[3] and shares about 50% amino acid identity with human NGF, NT-3, and NT-4/5. The structure of each NT contains: (1) a signal peptide following the initiation codon; (2) a pro-region containing an N-linked glycosylation site and a proteolytic cleavage site for furin-like pro-protein convertases, followed by the mature sequence; and (3) a distinctive three-dimensional structure containing two pairs of antiparallel β-strands and cysteine residues in a cystine knot motif. Mature NTs are noncovalently-linked homodimers with molecular weight about 28 kDa. Dimerization appears essential for NT receptor activation.

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BDNF Signaling

Each NT binds one or more high-affinity receptors (the trk receptors) (Kd ~ 10^{-11} M).[4] Trk proteins are transmembrane receptor tyrosine kinases (RTKs) homologous to other RTKs such as the epidermal growth factor (EGF) receptor and insulin receptor family.[5] Signaling by RTKs involves ligand-induced receptor dimerization and dimerization-induced trans-autophosphorylation.[6,7] Receptor autophosphorylation on multiple tyrosine residues creates specific binding sites for intracellular target proteins, which bind to the activated receptor via SH2 domains.[6] For the NT family, these target proteins have been shown to include PLCγ1 (phospholipase C), p85 (the noncatalytic subunit of PI-3 kinase), and Shc (SH2-containing sequence);[4] activation of these target proteins can then lead to a variety of intracellular signalling cascades such as the Ras-MAP (mitogen-activated protein) kinase cascade and phosphorylation of CREB (cyclic AMP response element binding protein).[8-11] Binding specificity is conferred via the juxtamembrane Ig-like domain of the extracellular portion of the receptor in the following pattern:[12] trkA is the high-affinity receptor for NGF (with low-affinity binding by NT-3 in some systems), trkB is the high-affinity receptor for BDNF and NT-4/5 with lower-affinity binding by NT-3, and trkC is the high-affinity receptor for NT-3.[5]

In addition to the high-affinity NT receptors, all of the NTs bind to the low-affinity NT receptor, designated $p75^{NTR}$ (Kd ~ 10^{-9} M).[13] $P75^{NTR}$ has a glycosylated extracellular region involved in ligand binding, a transmembrane region, and a short cytoplasmic sequence lacking intrinsic catalytic activity. It is related to proteins of the tumor necrosis factor (TNFR) superfamily. NT binding to $p75^{NTR}$ is linked to several intracellular signal transduction pathways, including nuclear factor-κB (NF-κB), Jun kinase and sphingomyelin hydrolysis.[14] $P75^{NTR}$ signaling mediates biologic actions distinct from those of the high-affinity trk receptors, notably the initiation of programmed cell death (apoptosis) as well as newly-described roles in the regulation of axonal elongation and synaptic transmission.[15]

Ligand-induced receptor tyrosine phosphorylation is necessary for NT-induced cellular responses.[5] For example, cooperative interaction between tyrosines in trkA mediates the neurite outgrowth effect of NGF.[16] Thus, receptor tyrosine phosphorylation seems a logical measure of the biologic level of NT activity (see below). Tyrosine-490 is phosphorylated following NT application and is known to couple trk receptors to Shc binding and activation of the ras-MAP kinase cascade.[11] Furthermore, recent evidence indicates that activated trk receptors may be endocytosed and retrogradely transported while still tyrosine phosphorylated.[17-23]

Localization, Transport and Release of BDNF

BDNF mRNA as well as the mRNA encoding the high-affinity receptor for BDNF (trkB) has a widespread distribution in the central nervous system, especially in the cerebral cortex, hippocampal formation, and amygdaloid complex.[24-26] Notably, high levels of BDNF and trkB expression are found in brain areas that have been associated with seizure susceptibility, such as hippocampus and entorhinal cortex.[27] Within hippocampus, the granule cells, pyramidal cells, and some hilar GABAergic neurons express mRNA for BDNF and trkB.

In parallel, BDNF protein immunoreactivity is also widespread, and appears to be localized in neuronal cell bodies, axons and dendrites.[24] Like BDNF mRNA, constitutive BDNF protein expression is high in the hippocampus, where the mossy fiber axons of dentate granule cells are intensely immunoreactive for BDNF.[24,28]

Unlike the classical target-derived trophic factor model in which NTs—such as NGF—are retrogradely transported, there is now abundant evidence that BDNF is also anterogradely transported in brain.[24,29-33] Indeed, a recent study using green fluorescent protein (GFP)-tagged BDNF demonstrated direct activity-dependent transneuronal transfer of BDNF to postsynaptic neurons.[34,35] In hippocampus, it appears that BDNF within the hilus and CA3 stratum lucidum is synthesized by the dentate granule cells, anterogradely transported and preferentially stored in mossy fiber terminal boutons.[36]

Biochemical studies suggest that endogenous BDNF may be packaged in a releasable vesicular pool[37] and recent evidence indicates that NTs are released acutely following neuronal depolarization in an intracellular calcium and phospholipase C (PLC)-dependent manner.[38-42]

BDNF Effects in Development

NTs are known to have profound survival, differentiation, and morphoregulatory effects during brain development (leading to formation of appropriately matched functional circuitry).[43-45] The classical view of NT function derived initially from studies of NGF includes effects on growth and survival of neurons, and indeed BDNF has also been shown to be necessary for the survival of some neurons during vertebrate development. Certain peripheral sensory neurons, especially those in vestibular and nodose-petrosal ganglia, depend on the presence of BDNF because BDNF knockout mice (lacking both alleles for BDNF) demonstrate loss of these sensory neurons.[45,46] Unlike NGF, however, sympathetic neurons are not affected, nor are motor neuron pools. BDNF knockout mice fail to thrive, demonstrate lack of proper coordination of movement and balance, and ultimately die by 3 weeks of age. Conversely, provision of BDNF or other NTs to peripheral nerves during development enhances outgrowth,[47] and can support and/or rescue certain sensory neurons.[48,49]

BDNF expression increases in the early postnatal period and then stays high into adulthood, consistent with a role in the mature CNS as well. In vitro and in vivo studies have demonstrated that BDNF has survival- and growth-promoting actions on a variety of CNS neurons, including hippocampal and cortical neurons. Lack or blockade of BDNF leads to death of certain identified forebrain neurons. For example, lack of cortical BDNF leads to death of dorsal thalamic neurons.[50] Similarly, deletion of trkB leads to loss of neocortical neurons.[51]

In addition to its effects on survival, BDNF appears to regulate neuronal morphology and synaptogenesis. BDNF has been shown to enhance axonal branching in cultures of hippocampal neurons[52,53] and also has been shown to have significant differential effects on dendritic branching in cortex[54-56]. Evidence that activity-induced NT expression may modulate axonal sprouting in vivo comes from modulation of retinotectal axon branching by BDNF;[57] inhibition of normal ocular dominance column formation by NT infusion[58,59] or trkB-Fc infusion;[60] and inhibition of pilocarpine-induced cholinergic sprouting in hippocampus by NGF antisera.[61]

BDNF Gene Regulation

A multitude of stimuli have been described that alter BDNF gene expression in both physiologic and pathologic states. Physiologic stimuli are known to increase BDNF mRNA content. For example, light stimulation increases BDNF mRNA in visual cortex,[62] and osmotic stimulation increases BDNF mRNA in the paraventricular hypothalamic nucleus.[63] Other naturalistic behaviors in animals increase BDNF mRNA expression. For example, whisker stimulation increases BDNF mRNA expression in rodent somatosensory barrel cortex;[64] and singing stimulates BDNF expression in the high vocal center (HVC) of adult male canaries.[65] Electrical stimuli that induce long-term potentiation (LTP) in the hippocampus, a cellular model of learning and memory, increase BDNF and NGF expression.[66-68] Even physical exercise has been shown to increase NGF and BDNF expression in hippocampus.[69,70]

This physiologic alteration in BDNF gene expression may be very important in the development of the brain. For example, there is an exciting body of work implicating BDNF in activity-dependent development of the visual cortex.[71] Provision of excess NGF[58] or BDNF[59] or blockade of BDNF signaling[60] leads to abnormal patterning of ocular dominance columns during a critical period of visual cortex development. This suggests a role for BDNF in the patterning of axonal arborizations from the lateral geniculate nucleus (LGN) to the visual cortex during development.

BDNF, Synaptic Plasticity, and Learning

A great deal of evidence now indicates that BDNF and its high-affinity receptor trkB, in addition to modulating neuronal survival and differentiation, are also critically involved in neuronal excitability[72] and modulation of synaptic transmission.[73-75] For example, application of NTs including BDNF has been shown to potentiate synaptic transmission in vitro[76-82] and in vivo.[83] BDNF enhances excitatory (glutamatergic) synaptic transmission[76,79] and reduces inhibitory (GABAergic) synaptic transmission.[84,85] In the hippocampus, a critical level of BDNF/trkB activation appears to be vital for modulation of synaptic efficacy. Incubation of hippocampal or visual cortical slices with the BDNF scavenger trkB-Fc reduces LTP,[86,87] and hippocampal slices from BDNF knockout animals exhibit impaired LTP induction which is restored by reintroduction of BDNF.[88-90] In addition, antagonists such as K252a block hyperexcitability in hippocampus due to BDNF exposure in vitro.[91]

The site and mechanism of synaptic potentiation by BDNF is not yet clear, but could involve facilitation of transmitter release,[92] phosphorylation of specific NMDA receptor subunits,[93] and/or direct effects on ion channels and conductances.[94-96] Enhanced excitatory transmission may also arise indirectly, because BDNF is known to have effects on the structure and function of inhibitory (GABAergic) neurons.[97] Reduction of trkB has been shown to reduce the ability of tetanic stimulation to induce LTP.[98] A recent study using imaging of dentate granule cells in mouse hippocampal slices identified BDNF-evoked calcium transients in dendritic spines but not at presynaptic sites, suggesting a postsynaptic site for BDNF-induced synaptic potentiation.[99,100]

Learning and memory depend on persistent selective modification of synapses between CNS neurons. Since BDNF appears to be critically involved in activity-dependent synaptic strengthening of the sort observed in the LTP model, there is great interest in its role as a molecular mechanism of learning and memory.

The hippocampus, which is required for many forms of long-term memory in humans and animals, appears to be an important site of BDNF action. Indeed, there is rapid and selective induction of BDNF expression in the hippocampus during contextual learning.[101] In addition, tool-use learning increases BDNF mRNA in monkey parietal cortex.[102] Function-blocking antibodies to BDNF, BDNF knockout,[103] antisense oligonucleotides to BDNF[104] and/or knockout of forebrain trkB signaling in mice[105] impairs spatial learning.

BDNF and Disease

Pathologic states are also associated with alteration in BDNF gene expression. In neurodegenerative diseases, inadequate trophic support may be partially responsible. In conditions such as epilepsy and chronic pain sensitization, excessive activation of excitatory synaptic plasticity may contribute to the disease phenotype.

BDNF and Epilepsy

Epilepsy is a disorder of the brain characterized by periodic and unpredictable occurrence of seizures. Although complex partial epilepsy is the most common type of epilepsy in adults (40% of all cases),[106] seizure control is achieved in only 25% of adults. It is clear that complex partial epilepsy is a major public health problem in that approximately 1 million people in the United States are affected and sufferers experience the periodic and unpredictable occurrence of seizures leading to impairment of consciousness. This handicap severely impairs the performance of many tasks and secondarily the procurement and maintenance of steady employment.

Elucidating the mechanisms of epileptogenesis in cellular and molecular terms may provide novel therapeutic approaches. Seizures have been shown to stimulate the expression of a variety of genes including those encoding transcription factors,[107,108] neuropeptides,[109] GAP-43,[110] proteases[111] and, quite prominently, NTs and trk receptors. The discovery that limbic seizures increase mRNA levels for nerve growth factor[112] led to the idea that seizure-induced expression

of neurotrophic factors may contribute to the lasting structural and functional changes underlying epileptogenesis.[27,113] Indeed, recent in vitro and in vivo findings implicate BDNF in the cascade of electrophysiologic and behavioral changes underlying the epileptic state.[114]

In particular, BDNF, NGF and trkB mRNA levels are increased in kindling and other seizure models whereas NT-3 mRNA content is decreased.[25,109,113,115-125] The magnitude of increase is greatest for BDNF in the hippocampus with BDNF mRNA being markedly upregulated in the dentate gyrus and CA1-CA3 pyramidal cell layers.[27,124]

This mRNA upregulation is accompanied by protein upregulation as well; extracts and in vivo microdialysates from animals after chemical convulsions show marked increases in neurotrophic factor activity.[126,127] Increases in BDNF protein content have been described following hilar lesion-induced limbic seizures, kindling and kainate administration.[128-131]

Seizure-induced increases in BDNF mRNA levels are transient compared to a longer-lasting increase in BDNF protein content. For example, following lesion-induced recurrent limbic seizures, BDNF mRNA levels peak 6 hours after seizure onset and return to control levels by about 12 hours;[113] in contrast, initial increases in BDNF protein content lag behind mRNA changes by 4 hours but remain well elevated over 4 days after the seizure episode.[128]

Following seizures, newly expressed BDNF appears to be anterogradely transported. Using hippocampal microdissection and quantification of BDNF by two-site ELISA, Elmer et al showed that BDNF protein levels after seizures were maximal at 12 hours in the dentate gyrus but at 24 hours in CA3,[129] consistent with anterograde transport of seizure-induced BDNF protein. Other evidence indicates that there is increased BDNF immunoreactivity in dentate granule cells by 4 hours followed by large increases in hilus and CA3 stratum lucidum at 12-24 hours; at the latter time point BDNF immunoreactivity within the granule cell bodies had returned to control levels.[132]

Effects of Inhibition of BDNF/trkB in Seizure Models

Recent studies using the kindling model of epilepsy have functionally implicated BDNF in epileptogenesis. In the kindling model, repeated, focal application of initially subconvulsive electrical stimuli eventually results in intense focal and tonic-clonic seizures.[133-136] Once established, this enhanced sensitivity to electrical stimulation persists for the life of the animal. The kindling model has been an important tool, since it allows experimental control over seizures and precise quantitation of effects of experimental manipulation on epileptogenesis in vivo.

Funabashi et al[137] and Van der Zee et al[138] found that kindling development was delayed by intraventricular infusion of anti-NGF antisera; however, the lack of specificity of the antisera limited interpretation of these experiments. Kokaia et al[139] reported a significant reduction in the rate of kindling development in BDNF heterozygous (+/-) mutant mice. Both basal and seizure-induced levels of BDNF mRNA were lower in the BDNF +/- compared to wild-type mice. The two-fold reduction in kindling rate in these animals is striking given that there was presumably some reduction but not elimination of trkB receptor signaling. Conversely, transgenic mice overexpressing BDNF display increased seizure severity in response to kainic acid and some display spontaneous seizures.[140] Infusion of BDNF itself into the hippocampus of adult rats leads to spontaneous limbic seizures as well as decreased threshold to chemoconvulsant-induced status epilepticus.[141] Of course, results from both the embryonic BDNF +/- knockouts and the BDNF transgenic mice must be cautiously interpreted in light of potential developmental effects of altered BDNF levels. The availability of conditional knockouts for trkB will enable analysis of the importance of trkB signaling in adult animals de novo.[51]

Recently, we attempted to selectively block trkB receptors during kindling development using trk-specific 'receptor bodies'.[142] These compounds are divalent homodimers that contain the ligand-binding domain of a given trk receptor and thus act as false receptors or 'receptor bodies' that putatively sequester endogenous NT (Figs. 1A, 1B). Intracerebroventricular (ICV) infusion of trkB receptor body (trkB-Fc) inhibited development of kindling in comparison to saline or human IgG controls, trkA-Fc, or trkC-Fc[142] (Figs. 2A, 2B). This effect manifested as

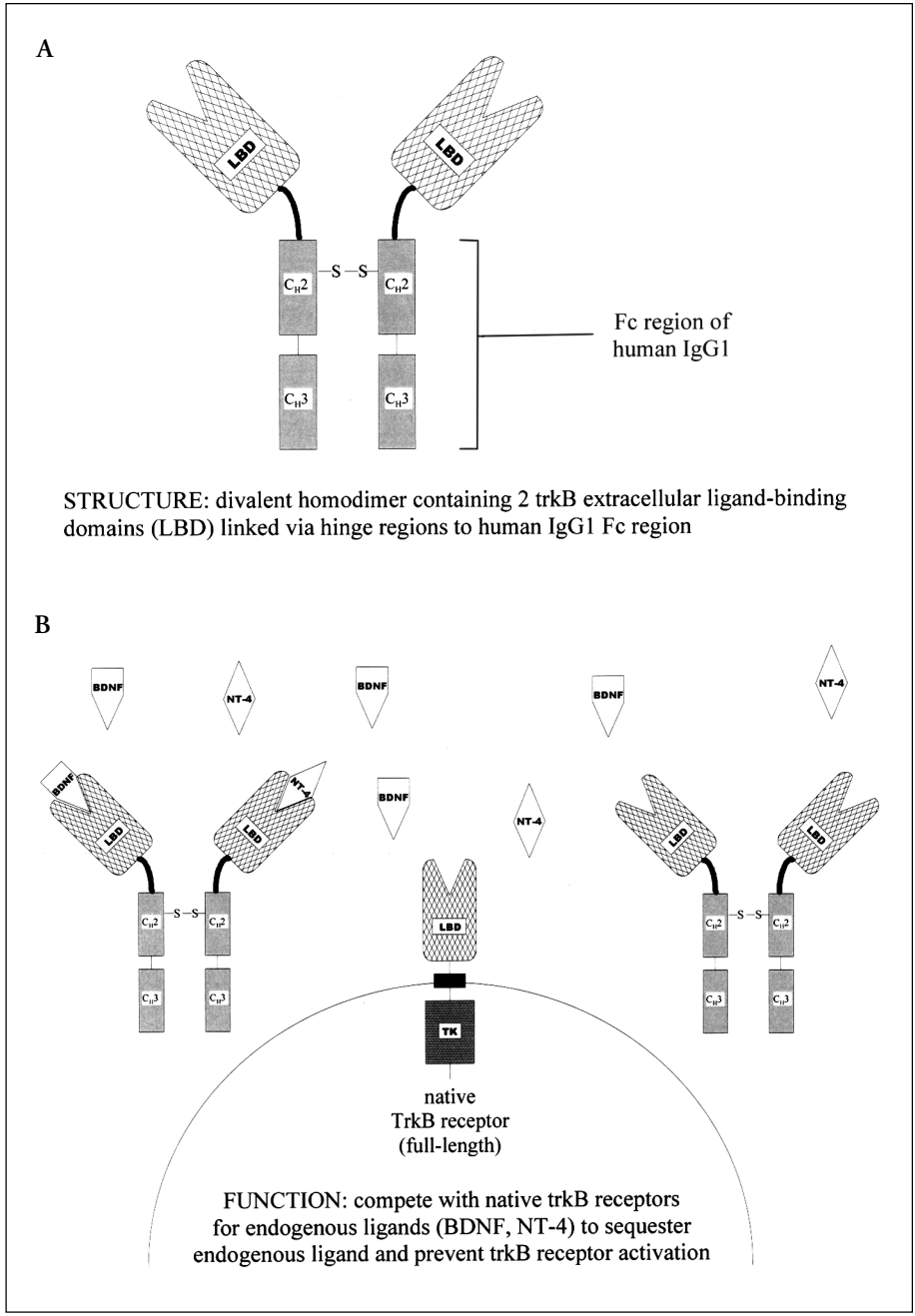

Figure 1. Schematic of structure and function of trkB receptor bodies.

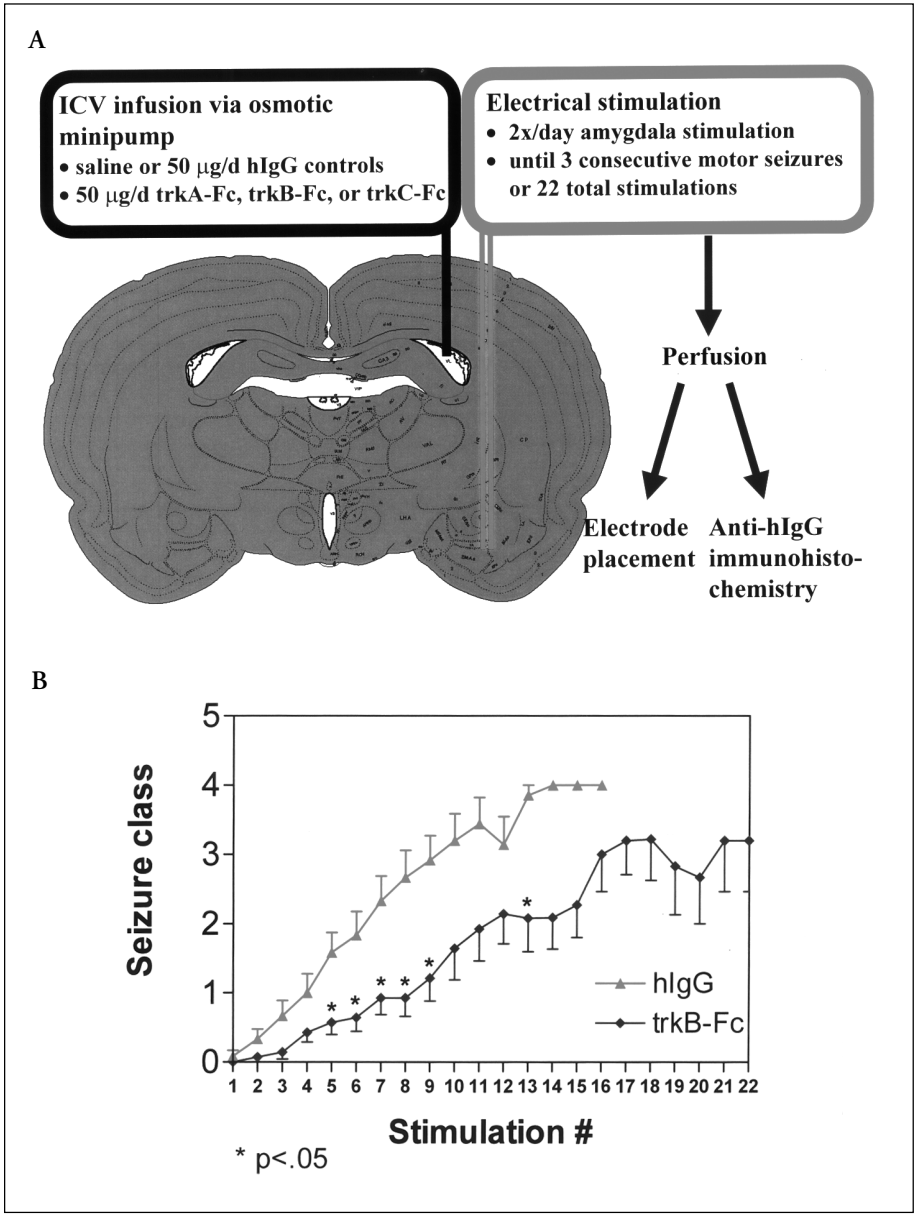

Figure 2A-D. TrkB receptor body inhibits kindling development. For details see Binder et al.[142]
A) Experimental design of ICV protein administration and kindling. B) TrkB-Fc inhibits kindling development compared to human IgG. Reprinted with permission from ref. 142, copyright 1999 Society for Neuroscience.

a reduction in behavioral seizure intensity during kindling development (Fig. 2C). Furthermore, we found that the degree of immunohistochemical penetration of trkB-Fc into hippocampus, but not striatum, septum or other structures correlated with the magnitude of inhibition of kindling development (Fig. 2D).[142]

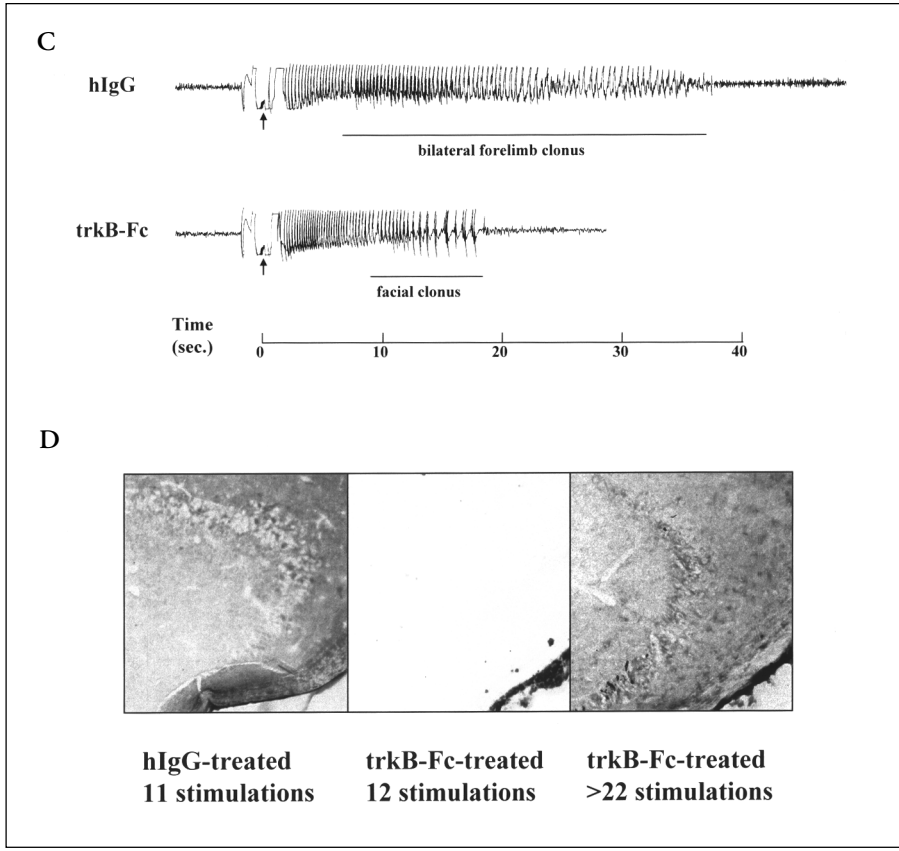

Figure 2, cont'd. C) Representative electroencephalograms from animals at kindling stimulation #15 from human IgG vs. trkB-Fc. Seizure duration and seizure intensity are decreased in trkB-Fc-treated animal. Reprinted with permission from ref. 142, copyright 1999 Society for Neuroscience. D) Hippocampal Fc immunoreactivity correlates with inhibition of kindling by trkB-Fc. Presence of Fc immunoreactivity indicating hippocampal penetration of trkB-Fc correlates with inhibition of kindling development (>22 vs. 12 stimulations to reach kindling criterion).

The finding that ICV trkB-Fc interferes with kindling suggests that BDNF and trkB signaling contributes to the development of kindled seizures. In apparent conflict with these findings, chronic intrahippocampal infusion of BDNF inhibits hippocampal kindling development and reduces electrographic seizure duration.[143] However, prolonged exposure to increased concentrations of BDNF suppresses trkB receptor responsiveness and reduces trkB mRNA and protein levels in vitro;[144,145] likewise, a six-day infusion of BDNF into the adult hippocampus in vivo decreases levels of full-length trkB receptor by 80%.[144] Thus, it is likely that chronic BDNF infusion in these kindling studies led to trkB downregulation and reduced responsiveness; if so, the retarded kindling development observed is consistent with the findings of the trkB-Fc infusion studies and those of BDNF heterozygotes[139] in implicating trkB receptor activation in kindling development. Alternatively, BDNF infusion could have upregulated the inhibitory neuropeptide Y (NPY) in these studies (see below).

Epileptogenesis in transgenic mice overexpressing the truncated form of trkB, a dominant negative receptor for BDNF, has recently been examined.[146] After kainic acid-induced status epilepticus, development of spontaneous seizures was monitored by video-EEG. This study

demonstrated that transgenic mice expressing truncated trkB (which would presumably downregulate BDNF signaling through the full-length catalytic trkB receptor) had a lower frequency of spontaneous seizures, and had less severe seizures with later onset and lower mortality.[146]

Activation of trk Receptors after Seizures

The above work suggests that limiting activation of the trkB receptor inhibits epileptogenesis, but this does not address whether or where NT receptor activation occurs during epileptogenesis. Since ligand-induced receptor tyrosine phosphorylation is required for NT-induced cellular responses,[5] receptor tyrosine phosphorylation seems a logical measure of the biologic level of NT activity. Using antibodies that selectively recognize the phosphorylated form of trk receptors (Fig. 3A), we found that in contrast to the low level of phosphotrk immunoreactivity constitutively expressed in the hippocampus of adult rats, phosphotrk immunoreactivity was strikingly increased following partial kindling or kainate-induced seizures.[147] Following seizures, phosphotrk immunoreactivity was selectively increased in dentate hilus and CA3 stratum lucidum of hippocampus (Fig. 3B). This distribution coincides with the 'mossy fiber' pathway arising from the dentate gyrus granule cells (Fig. 3C). This immunoreactivity could be selectively competed with phosphotrk peptide (Fig. 3D).

Interestingly, the anatomic distribution, time course and threshold for seizure-induced phosphotrk immunoreactivity corresponds well to the demonstrated pattern of BDNF upregulation by seizures. That is, both phosphotrk and BDNF immunoreactivities are most prominently increased in hippocampal CA3 stratum lucidum and maximally increased at 24 hours after seizure onset (Fig. 3E).[147] This suggests that the phosphotrk immunoreactivity may be caused by seizure-induced increases in BDNF expression and release. Taken together with the kindling data, these results imply that activation of trkB receptors contributes to the development of kindling, and implicate the hippocampus and in particular the mossy fiber-CA3 synapse as a primary site of trkB action.

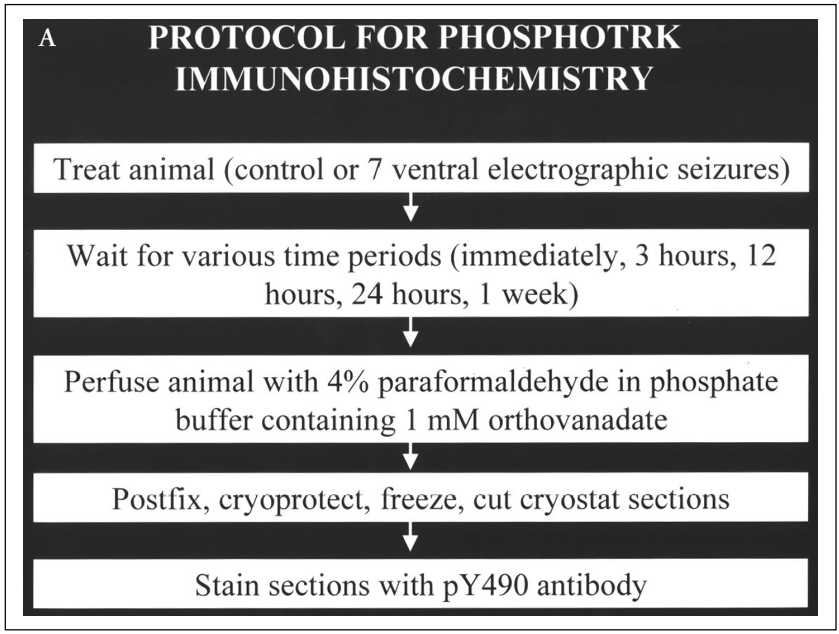

Figure 3. Seizures induce trk phosphorylation in the mossy fiber pathway of adult rat hippocampus. For details see Binder et al.[147] A) Protocol for phosphotrk immunohistochemistry.

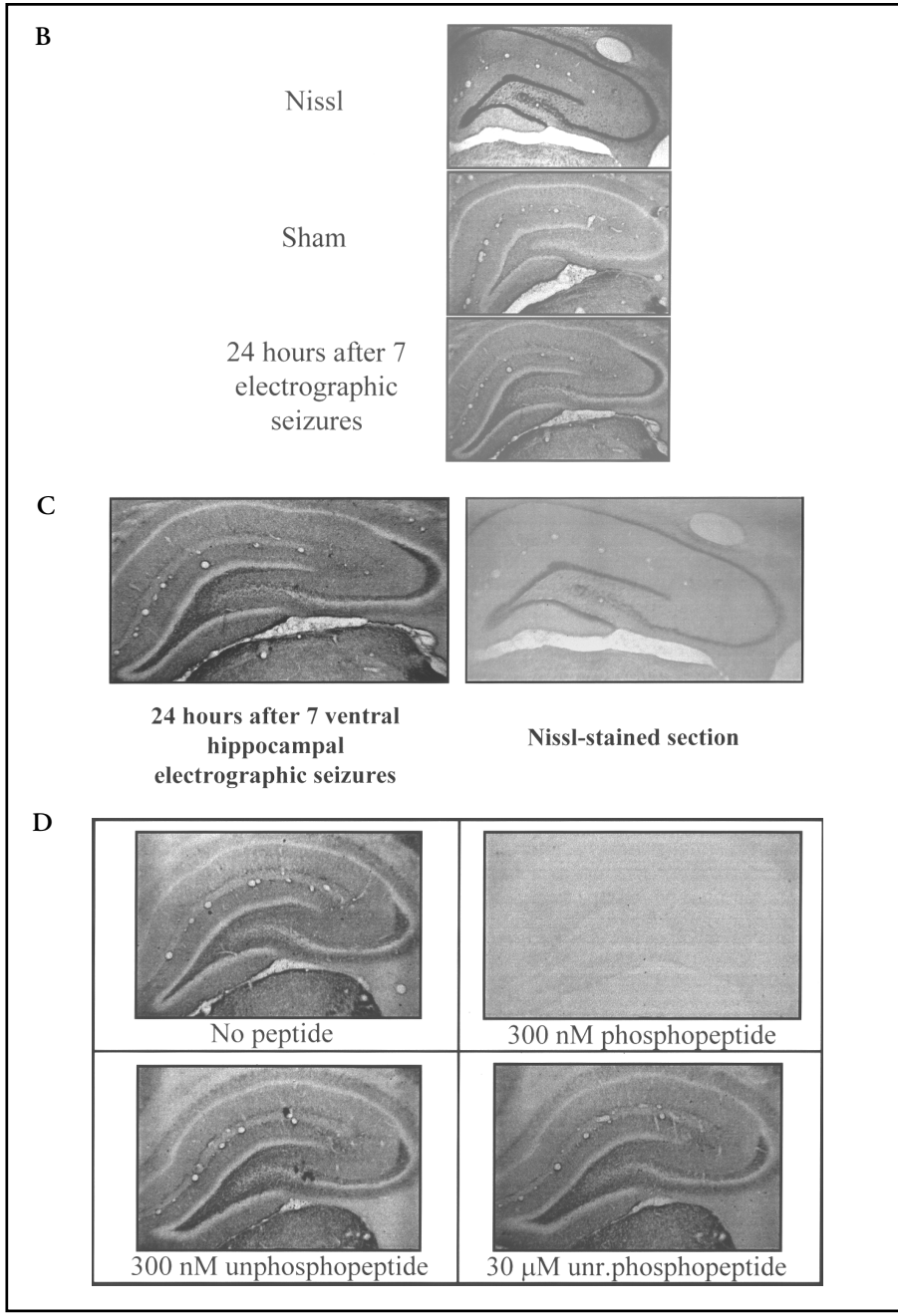

Figure 3, cont'd. B) Seizures increase phosphotrk immunoreactivity in hilus and CA3 stratum lucidum. B-E reprinted with permission from ref. 147, copyright 1999 Society for Neuroscience. C) Comparison of distribution of phosphotrk immunoreactivity with Nissl-stained section demonstrating mossy fiber pathway localization of phosphotrk immunoreactivity. D) Peptide competition of phosphotrk immunoreactivity. Sections are from a rat 24 hours after 7 electrographic seizures.

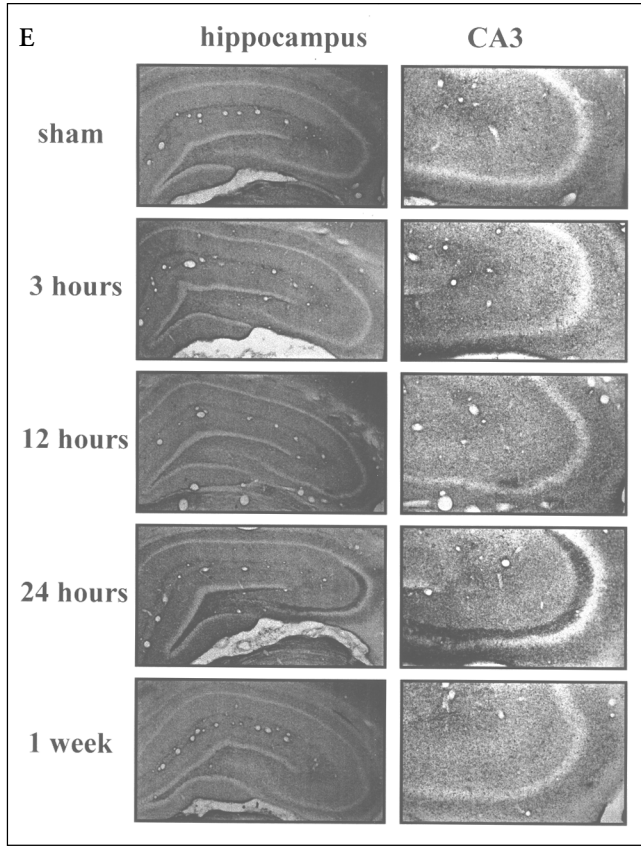

Figure 3, cont'd. E) Time course of phosphotrk immunoreactivity following 7 ventral hippocampal seizures (left: hippocampus, right: CA3). Note phosphotrk immunoreactivity in hilus and CA3 stratum lucidum at the 24-hour time point.

BDNF-Induced Hyperexcitability of the Mossy Fiber-CA3 Synapse

Based on the above data, one might speculate that BDNF upregulation in the adult brain could predispose certain areas to seizures. Indeed, in adult rat hippocampal slices BDNF exposure can produce multiple discharges and spreading depression in area CA3 and the entorhinal cortex upon afferent stimulation.[91] Acute application of exogenous BDNF to hippocampal slices appears to preferentially enhance the efficacy of the excitatory mossy fiber synapse onto CA3 pyramidal cells.[91]

Actions of BDNF have also been examined after pilocarpine-induced status epilepticus and chronic seizures, when sprouting of mossy fiber collaterals occurs. The new collaterals innervate processes in the inner molecular layer, including granule cell dendrites.[148] In hippocampal slices isolated from pilocarpine-treated rats, BDNF enhances responses to stimulation of the mossy fiber collaterals recorded in the inner molecular layer.[149] These effects can be blocked by K252a, a trk inhibitor, and confirm a preferential enhancement of mossy fiber synaptic transmission by BDNF. In addition, BDNF exposure in these epileptic animals led to seizure-like events.[149] Consistent with this are the observations of heightened seizure susceptibility, spontaneous seizures, and hyperexcitability of hippocampal field CA3 in BDNF-overexpressing transgenic mice.[140]

Cellular Model of BDNF-trkB Interaction

The studies summarized above indicate that upregulation of BDNF mRNA, protein and receptor activation occurs during epileptogenesis, that this upregulation is functionally relevant to increased excitability, and that the hippocampus and closely associated limbic structures may be particularly important in the pro-epileptogenic effects of BDNF. A cellular and molecular model of the actions of BDNF in promoting excitability in the hippocampus follows from these studies (Fig. 4). BDNF mRNA upregulation by seizure or perhaps by other stimuli such as ischemia or traumatic brain injury leads to increased BDNF production by the dentate granule cells, heightened anterograde transport and release of BDNF from mossy fiber axons and activation of trkB receptors in hilus and CA3 stratum lucidum. The locus of activation of trkB receptors by released BDNF may be either pre- or postsynaptic.[150,151] TrkB receptor activation could lead to acute depolarization,[96] enhanced glutamatergic synaptic transmission,[79,92] or reduced inhibitory synaptic transmission.[84] Recent data based on the LTP model suggest that BDNF's actions may be primarily postsynaptic.[100] These alterations in synaptic transmission, either alone or in combination with other changes (see below) could be sufficiently long-lived to underlie a permanent hyperexcitability of the hippocampal network (i.e., the epileptic state).

The relevance of results implicating BDNF in modulation of synaptic transmission to epileptogenesis depends critically on whether such modulation occurs in epileptic tissue. Several lines of evidence suggest this is the case. First, BDNF expression is increased in hippocampi of patients with temporal lobe epilepsy (see below). Second, evidence for modulation of ionotropic receptors with epilepsy comes from studies demonstrating altered electrophysiology of dentate granule cells in kindling,[152,153] other animal models [154-156] and human epileptic tissue.[157] Third, increased excitability of CA3 pyramidal cells is observed in kindled animals as detected by increased epileptiform bursting induced by elevated K^+ or lowered Mg^{++} in isolated hippocampal slices.[158,159] CA3 excitability is also present in other animal models.[160] Fourth,

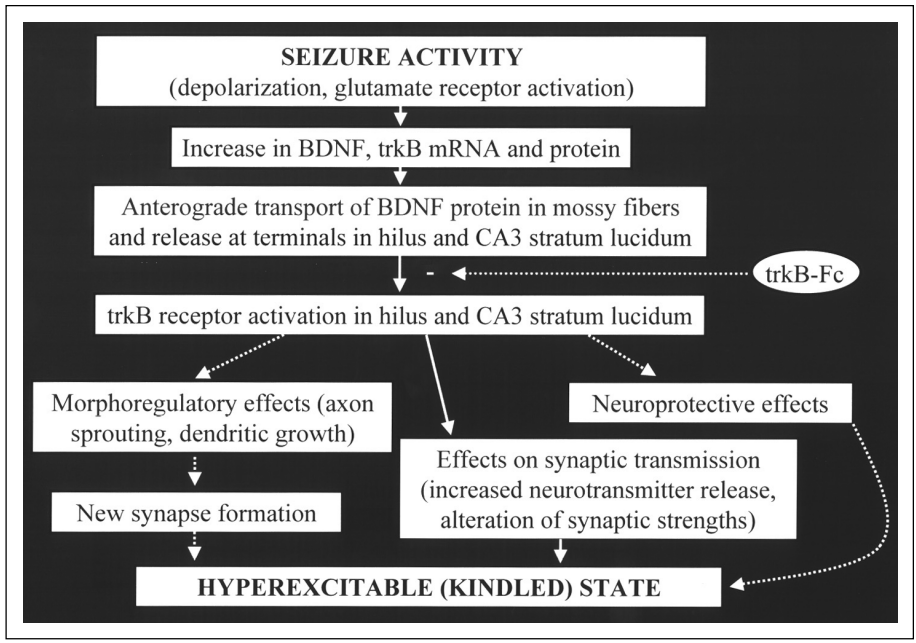

Figure 4. Model of BDNF/trkB involvement in epileptogenesis.

tetanic stimulation of the mossy fiber pathway in hippocampal slices (such as might occur during a seizure) induces synaptic potentiation onto CA3 pyramidal cells while inducing depression onto stratum lucidum interneurons.[161]

While modulation of multiple synaptic stations in the limbic system probably contributes to hyperexcitability following seizures, the pivotal role of the CA3 pyramidal cells in promoting epileptiform activity in the hippocampus; the role of BDNF in hippocampal synaptic transmission; the fact that constitutive and seizure-induced BDNF immunoreactivity within the hippocampus is most intense in the mossy fiber pathway;[24,28] together with the localization of seizure-induced trk receptor activation in CA3 stratum lucidum[147] all suggest that strengthening of the excitatory mossy fiber input onto CA3 pyramidal cells may be a primary mechanism by which BDNF promotes epilepsy.

Other Effects of BDNF

Based on the known effects of BDNF, it is possible that trkB receptor activation could contribute to epileptogenesis not only via synaptic effects on excitability but also by inducing changes in dendritic or axonal sprouting, synaptic morphology and synapse formation on a slower time scale. The most prominent synaptic reorganization known to occur in the epileptic brain is sprouting of the dentate granule cell mossy fibers.[162] Interestingly, mossy fiber sprouting was increased in BDNF +/- compared to +/+ mice despite the inhibition of kindling development in these mutants.[139] In addition, bath-applied trkB-Fc failed to inhibit kainate-induced mossy fiber sprouting in hippocampal explant cultures.[163] BDNF-overexpressing transgenic mice failed to demonstrate increased mossy fiber sprouting.[164] Thus, there is little evidence to date to suggest that BDNF upregulation is responsible for mossy fiber sprouting in the adult brain during epileptogenesis.

BDNF is known to modulate the expression of neurotransmitters and neuropeptides, many of which have potential roles in seizures. Perhaps the best characterized of these is neuropeptide Y (NPY). BDNF but not NGF is known to increase NPY levels.[165] NPY is thought to be inhibitory to seizure generation because NPY knockout animals exhibit increased seizure susceptibility.[166] Interestingly, both kindling and kainate-induced seizures increase NPY immunoreactivity in the mossy fibers[131,167] in a distribution strikingly similar to that of phosphotrk immunoreactivity. This suggests that BDNF-induced trk activation may lead to NPY upregulation in an overlapping anatomic distribution, thereby potentially limiting excitability.[143]

BDNF and Human Epilepsy

Animal models of epilepsy, in particular the kindling model described above, have implicated BDNF in epileptogenesis. What direct evidence is there that BDNF is altered/involved in human epilepsy?

Mathern et al found increased expression of BDNF mRNA in dentate granule cells from patients with temporal lobe epilepsy.[168] Similarly, Murray et al performed in situ hybridization for BDNF mRNA in resected temporal lobe epilepsy specimens and found increased hippocampal BDNF expression compared to autopsy control tissue.[169] Takahashi et al recently showed that protein levels of BDNF but not other NTs were upregulated 2.6-fold in human epilepsy tissue specimens.[170] Interestingly, this study also demonstrated a corresponding upregulation of hippocampal NPY. Recently, Zhu and Roper recorded from hippocampal slices from patients with temporal lobe epilepsy and found that BDNF application enhanced fast excitatory transmission in dentate granule cells.[171]

BDNF may also be involved in tumor-associated epilepsy. Primary brain tumors are often associated with seizures.[172] Interestingly, immunohistochemical expression of BDNF and trkB have been studied in glioneuronal brain tumors. In a study of 40 patients with gangliogliomas and 15 patients with dysembryoplastic neuroepithelial tumors (DNETs), tumors that are associated with chronic medically intractable epilepsy, Aronica et al have recently shown that there is intense immunoreactivity for both BDNF and trkB in these tumors, and furthermore this

immunoreactivity is colocalized with NMDAR1 immunoreactivity suggesting a functional interaction potentially contributing to the epilepsy associated with these lesions.[173]

BDNF and Other Diseases of the Adult Nervous System

BDNF and Neurodegenerative Diseases

The idea that degenerative diseases of the nervous system may result from insufficient supply of neurotrophic factors has generated great interest in BDNF as a potential therapeutic agent. Many reports have documented evidence of decreased expression of BDNF in Alzheimer's and Parkinson's disease.[174] Selective reduction of BDNF mRNA in the hippocampus has been reported in Alzheimer's disease specimens and decreased BDNF protein has been demonstrated in the substantia nigra in Parkinson's disease, areas that degenerate in these diseases. BDNF promotes survival of all major neuronal types affected in Alzheimer's and Parkinson's disease, such as hippocampal and neocortical neurons, cholinergic septal and basal forebrain neurons, and nigral dopaminergic neurons. Interestingly, recent work has implicated BDNF in Huntington's disease as well. Huntingtin, the protein mutated in Huntington's disease, upregulates BDNF transcription, and loss of huntingtin-mediated BDNF transcription leads to loss of trophic support to striatal neurons which subsequently degenerate in the hallmark pathology of the disorder.[175] In all of these disorders, provision of BDNF or increasing endogenous BDNF production may conceivably be therapeutic if applied in the appropriate spatiotemporal context.

BDNF and Pain Transmission

BDNF also appears to play an important neuromodulatory role in pain transduction.[176] In particular, BDNF acts as a neuromodulator in small-diameter nociceptive neurons.[177] BDNF is synthesized, anterogradely transported and packaged by these neurons into dense core vesicles at nociceptor (C-fiber) terminals in the dorsal horn, and is markedly upregulated in inflammatory injury to peripheral nerves (along with NGF). Postsynaptic cells in this region express trk receptors,[178] and application of BDNF sensitizes nociceptive afferents and elicits hyperalgesia.[179]

An example of pathologic activity-dependent plasticity somewhat similar to epilepsy is central pain sensitization.[180,181] Central pain sensitization is an activity-dependent increase in excitability of dorsal horn neurons leading to a clinically intractable condition termed 'neuropathic pain' in which normally nonpainful somatosensory stimuli (touch and pressure) become exquisitely painful (allodynia). Like kindling and drug sensitization and dependence, central sensitization is NMDA receptor-dependent and long-lasting.[180]

Furthermore, as in kindling, NTs have been implicated in central sensitization.[182] BDNF is upregulated in dorsal root ganglia and spinal cord following peripheral inflammation.[183] In addition, BDNF reduces $GABA_A$-mediated currents in peripheral afferent fibers, suggesting that it may facilitate nociceptive input into the dorsal horn.[184] Woolf and colleagues have demonstrated that pretreatment with trkB-Fc prevents central sensitization, presumably by competing with endogenous BDNF released at C-fiber terminals onto dorsal horn neurons, thereby preventing activation of trkB receptors on dorsal horn membranes.[185] Inflammation-induced hyperalgesia also appears to be related to NGF/trkA signaling since trkA-Fc or NGF antibodies inhibit the development of hyperalgesia following an inflammatory stimulus.[186-188]

BDNF and Drug Addiction

The neurobiology of drug addiction is rapidly becoming better understood.[189] Emerging evidence indicates that BDNF-related plasticity may also occur in brain structures responsible for drug sensitization and dependence. For example, BDNF has been found to influence the reinforcing and locomotor activating properties of psychostimulants. Repeated injections of amphetamine lead to elevated BDNF mRNA expression in the basolateral amygdala, piriform cortex and paraventricular nucleus of the hypothalamus.[190] This is accompanied by increased

BDNF immunoreactivity in target structures such as the nucleus accumbens, a well-known site related to reinforcing behavior and addiction.[190]

Chronic opiate exposure leads to numerous neurochemical adaptations, in particular in the noradrenergic locus ceruleus (LC). Such adaptations are thought to contribute to physical drug dependence. Now, it appears that opiate administration and withdrawal lead to changes in BDNF expression. Numan et al demonstrate that whereas chronic morphine treatment results in only modest increases in BDNF in the locus ceruleus, withdrawal leads to a marked, rapid and prolonged increase in BDNF and trkB mRNA in the LC.[191] More recently, Akbarian et al[192] have shown that there are dramatic alterations of morphine administration-induced signaling in mice with a conditional deletion of BDNF in postnatal brain. In these mice, there was a three-fold reduction in opiate withdrawal symptoms.[192]

All drugs of abuse increase dopamine in the shell of the nucleus accumbens, and the D3 receptor is thought to be responsible for the reinforcing effects of drugs.[193] A recent study suggested a candidate molecular mechanism for the control of BDNF over behavioral sensitization. Guillin et al demonstrated that BDNF from dopaminergic neurons is responsible for inducing normal expression of the dopamine D3 receptor in nucleus accumbens.[194] Thus, pathologic alterations in BDNF expression may lead to the abnormal D3 expression seen in drug addiction.

BDNF and Affective Behaviors

BDNF signaling may also be involved in affective behaviors.[195,196] BDNF may be dysregulated in depressed individuals.[196] Environmental stresses such as immobilization that induce depression also decrease BDNF mRNA.[197] Conversely, physical exercise is associated with decreased depression and increased BDNF mRNA. Existing treatments for depression are thought to work primarily by increasing endogenous monoaminergic (i.e., serotonergic and noradrenergic) synaptic transmission, and recent studies have shown that effective antidepressants increase BDNF mRNA in the brain. Exogenous delivery of BDNF promotes the function and sprouting of serotonergic neurons in adult rat brains.[195] Thus, new pharmacologic strategies are focused on the potential antidepressant role of BDNF.

A recent study suggests that the BDNF gene may be a susceptibility gene for bipolar disorder, a severe psychiatric disease that affects 1% of the population worldwide and is characterized by recurrent bouts of mania and depression.[198] This study demonstrates linkage disequilibrium between two polymorphisms of the BDNF gene and bipolar disorder in 283 nuclear families.[198]

Summary

BDNF is widespread in the CNS during development and in adulthood, and is regulated in a wide variety of physiologic and pathologic states. Overall, in addition to its trophic effects on target neurons, BDNF appears to constitute a general mechanism for activity-dependent modification of synapses in the developing and adult CNS. Diseases of abnormal trophic support (such as neurodegenerative diseases) and diseases of abnormal excitability (such as epilepsy and central pain sensitization) can be related in some cases to abnormal BDNF signaling.

The evidence implicating BDNF in pathologic activity-dependent plasticity is most clear in the case of epilepsy. BDNF mRNA and protein are markedly upregulated in the hippocampus by seizure activity in animal models, and interference with BDNF/trkB signaling inhibits epileptogenesis. The hippocampus and closely associated limbic structures are thought to be particularly important in the pro-epileptogenic effects of BDNF, and indeed increased BDNF expression in the hippocampus is found in specimens from patients with temporal lobe epilepsy.

It is hoped that understanding of the hyperexcitability associated with BDNF may lead to novel anticonvulsant or antiepileptic therapies. Further study of the cellular and molecular mechanisms by which BDNF influences cell survival and excitability will likely provide novel concepts and targets for the treatment of diverse CNS diseases.

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