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New insights in the biology of BDNF synthesis and release: implications in CNS function

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Abstract

BDNF has pleiotrophic effects on neuronal development and synaptic plasticity that underlie circuit formation and cognitive function. Recent breakthroughs reveal that neuronal activity regulates BDNF cell biology, including *Bdnf* transcription, dendritic targeting and trafficking of BDNF mRNA and protein, and secretion and extracellular conversion of proBDNF to mature BDNF. Defects in these mechanisms contribute differentially to cognitive dysfunction and anxiety–like behaviors. Here we review recent studies, presented at a symposium at Neuroscience 2009, that describe regulatory mechanisms that permit rapid and dynamic refinement of BDNF actions in neurons.

Neurotrophins are central to many facets of CNS function, with critical roles in cell differentiation, neuronal survival, migration, dendritic arborization, synaptogenesis and activity-dependent forms of synaptic plasticity (Bramham et al., 2008; Cohen et al., 2008; Lu et al., 2008; Reichardt, 2006). Of the four neurotrophins, the actions of brain derived neurotrophic factor (BDNF) on central neurons have been best characterized. Indeed, modest changes in BDNF levels, or expression of a frequent non-synonymous single nucleotide polymorphism in the *Bdnf* gene are correlated with neuroanatomical differences and behavioral changes in humans and in mouse models, including abnormal feeding behavior, alterations in episodic memory, and susceptibility to neuropsychiatric disorders of anxiety and depression (Castren and Rantamaki, 2008; Chen et al., 2006; Egan et al., 2003; Lyons et al., 1999; Russo et al., 2009). These significant structural, cognitive and behavioral effects suggest that BDNF is highly regulated in vivo, with tight temporal, spatial and stimulus-specific expression. Indeed, BDNF levels are dynamically regulated in postnatal development, in part by activity-dependent mechanisms (Poo, 2001). Here we discuss four recently identified mechanisms that regulate *Bdnf* transcription and mRNA targeting, and BDNF protein processing and intracellular trafficking, that function to intricately regulate the expression and release of BDNF by neurons. Importantly, impairment in several of these distinct mechanisms leads to defined abnormalities in CNS circuitry and behavior, underscoring their biological importance.

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Activity Dependent BDNF Transcription in the Development of Cortical Inhibition

The *Bdnf* gene is comprised of at least eight distinct promoters that initiate transcription of multiple distinct mRNA transcripts, each of which contains an alternative 5' exon spliced to a common 3' coding exon that contains the entire open reading frame for the BDNF protein (Aid et al., 2007). Through the use of alternative promoters, splicing and polyadenylation sites, at least 18 transcripts can be produced, but remarkably, each encodes an identical initial BDNF protein product. Although the significance of this complex transcriptional organization is cryptic, one hypothesis is that it provides multiple layers of regulation, through alternative promoter usage, differential mRNA stability or differential subcellular localization of either mRNA or protein.

In the cortex, promoter IV-dependent *Bdnf* transcription accounts for the majority of the neuronal activity-induced B*dnf* expression (Tao et al., 2002; Timmusk et al., 1994). To determine which, if any, of the physiological functions of BDNF in the nervous system are specifically controlled by the neuronal activity-regulated component of its expression, a subtle knockin mutation was introduced into *Bdnf* promoter IV, and expressed in the cortex (Hong et al., 2008). The resulting animal exhibits disrupted sensory experience-dependent induction of *Bdnf* expression. Neurons from these animals form fewer inhibitory synapses and have fewer spontaneous inhibitory quantal events (Hong et al., 2008). Significantly, these animals exhibit reduced expression of inhibitory presynaptic markers in the cortex, indicating a specific requirement for activity-dependent *Bdnf* expression in the development of cortical inhibition. Interestingly, these animals do no exhibit altered survival or differentiation of GABAergic neurons, demonstrating a selective role for activity-dependent *Bdnf* expression in establishing appropriate connectivity in the central nervous system (Hong et al., 2008). The lack of other phenotypes associated with reduced BDNF expression, such as hyperphagia and obesity, in these animals highlights the ability to this approach to delineate promoter-specific effects, as well as activity-dependent effects on BDNF action.

Regulation of dendritic trafficking of *Bdnf* mRNA modulates synaptic plasticity and spine development

In addition to utilization of different promoters, *Bdnf* transcripts are polyadenylated at either of two alternative sites, leading to two populations of mRNAs: those with a short 3'UTR and those with a long 3'UTR (Timmusk et al., 1993). These pools of *Bdnf* mRNAs are localized to distinct subcellular compartments of neurons, with long 3'UTR *Bdnf* mRNA enriched in dendrites of cortical neurons when compared to total *Bdnf* mRNAs. This observation raises the possibility that the long 3'UTR targets one pool of *Bdnf* mRNA to dendrites. To investigate this hypothesis, an existing mouse strain ($Bdnf^{klox/klox}$) in which the long 3'UTR is disrupted was utilized (Gorski et al., 2003), and indeed few *Bdnf* mRNAs were present in dendrites of cortical and hippocampal neurons from these animals. These findings indicate that the short 3'UTR *Bdnf* mRNA is restricted to the soma, whereas the long 3'UTR *Bdnf* mRNA is also targeted to dendrites for local translation (An et al., 2008).

Numerous mRNA species have been localized to neuronal dendrites, including transcripts encoding cytosolic, cytoskeletal, integral membrane, and secreted proteins (Bramham, 2008). Although local translation of these dendritic mRNAs is required for lasting synaptic plasticity, the *in vivo* function of local protein synthesis remains largely unknown. Despite of the truncation of the long 3'UTR, $Bdnf^{klox/klox}$ mice still produce normal amounts of *Bdnf* mRNAs and protein in the brain (An et al., 2008), permitting the use of this animal model to dissect the *in vivo* function of dendritic BDNF synthesis. As anticipated, the level of BDNF

protein in *Bdnf*$^{klox/klox}$ hippocampal neurons was increased in the soma, but decreased in dendrites, suggesting that a portion of dendritic BDNF protein is from local translation of dendritic *Bdnf* mRNA. Interestingly, *Bdnf*$^{klox/klox}$ mice exhibited denser and thinner dendritic spines of CA1 pyramidal neurons and reduced hippocampal long-term potentiation (An et al., 2008). As application of BDNF to cultured neurons stimulates spine formation, the spine phenotype in *Bdnf*$^{klox/klox}$ mice highlights the effects of locally translated BDNF in dendrites.

Development of spines involves overproduction, growth and pruning, and spine density increases with age in early development, followed by a reduction to reach levels in mature animals. In humans, spine density increases dramatically during the first few months of postnatal life, however ~40% of synapses are later selectively eliminated Huttenlocher, 1979). Maturation and pruning of dendritic spines is dependent on sensory experience and has been implicated in the activity-dependent refinement of synaptic connections (Churchill et al., 2002). The abnormal spine morphology in *Bdnf*$^{klox/klox}$ mice likely results from a deficit in spine pruning, as spine density at 3 weeks of age is normal (An et al., 2008). These animals, however, fail to reduce spine density from 3 weeks to 2 months of age, as do wild-type mice. Since dendritic mRNAs are likely to remain dormant until stimulated, these observations suggest that neuronal activity may promote pruning and growth of dendritic spines by inducing local translation of dendritic *Bdnf* mRNA. Collectively, these studies suggest that in the *Bdnf* gene, specific 3'UTRs are utilized to direct *Bdnf* transcripts to dendrites, whereby locally translated BDNF may act to promote dendritic spine remodeling, in later postnatal development.

Regulation of BDNF action by altering conversion of proBDNF to mature BDNF

Although the above studies have focused upon mechanisms of regulation of *Bdnf* mRNA, BDNF actions can also be altered at the protein level. BDNF is initially synthesized as a precursor protein (preproBDNF) in endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF is transported to the Golgi for sorting into either constitutive or regulated secretory vesicles. ProBDNF may be converted into mature BDNF (mBDNF) intracellularly in the trans-Golgi by members of the subtilisin-kexin family of endoproteases such as furin, or in the immature secretory granules by pro-protein convertases (Mowla et al., 1999). It has long been thought that only secreted mBDNF is biologically active, and proBDNF is exclusively localized intracellularly, serving as an inactive precursor. However, recent observations of proBDNF secretion and its conversion to mBDNF in vitro by plasmin and matrix metalloproteases suggest that proBDNF may be biologically active (Pang et al., 2004), although the efficiency of intracellular cleavage is controversial (Matsumoto et al., 2008), and may vary among neuronal cell types.

BDNF and the tissue-Plasminogen Activator (tPA, a serine protease) have both been implicated in late phase long-term potentiation (L-LTP) and long-term memory. In vitro, proBDNF can be converted to mature BDNF by plasmin, through the tPA-dependent activation of plasminogen. In electrophysiological studies, mature BDNF, but not uncleavable proBDNF, rescues L-LTP in tPA and plasminogen knockout mice (Pang et al., 2004). These studies suggest that the conversion of proBDNF to mBDNF promotes L-LTP expression in the hippocampus, and provides a mechanistic link between tPA and BDNF, the only two secreted proteins implicated in long-term memory.

Proneurotrophins, including proBDNF, bind and activate the p75^{NTR} receptor (Teng et al., 2005). To determine if proBDNF alters synaptic activity, effects of recombinant proBDNF were evaluated in wild type and in p75^{NTR} mutant mice. Perfusion of uncleavable proBDNF

significantly enhances LTD, an effect not observed in $p75^{NTR}$ knockout animals (Woo et al., 2005). LTD is completely disrupted in juvenile (3–4 week) hippocampal slices from the $p75^{NTR}$ mutant mice. These results suggest that pro and mature BDNF elicit opposing synaptic effects through activation of two distinct receptors; $p75^{NTR}$ and Trk receptor tyrosine kinases. Interestingly, complete deletion of the *Bdnf* gene, which eliminates both pro and mature BDNF, did not impact LTD (Matsumoto et al., 2008), suggesting that complete deletion may obscure detection of potentially opposing phenotypes.

To better understand the mechanisms that regulate the conversion of proBDNF to mBDNF, experiments were designed to uncover physiologically relevant conditions under which the specific isoforms of endogenous BDNF are secreted. Under conditions that induce LTD (low-frequency stimulation or LFS), the majority of the secreted BDNF was proBDNF (Nagappan et al., 2009). In contrast, when the neurons were stimulated with high frequency (HFS – a condition that induces LTP), substantial amounts of secreted mBDNF were detectable. Interestingly, tPA, the protease involved in the extracellular conversion of proBDNF to mBDNF, was secreted only under HFS. Thus, both low- and high- frequency neuronal activities increased proBDNF in the extracellular milieu, but only high frequency neuronal activity induced tPA secretion resulting in extracellular conversion of proBDNF to mBDNF. These results demonstrate how high frequency neuronal activity may regulate opposing functions of BDNF isoforms.

The role of the proBDNF/$p75^{NTR}$ signaling and LTD in adult animals has also been evaluated *in vivo*. In the uninjured adult brain, $p75^{NTR}$ is almost exclusively expressed in basal forebrain cholinergic neurons, which project to the hippocampus. Upon exposure to acute stress, LTD can be induced in the adult hippocampus, in contrast to non-stressed adult animals which do not express LTD. Adult $p75^{NTR}$ knockout animals fail to express LTD in this paradigm, suggesting that $p75^{NTR}$ plays a central role. Interestingly, the $p75^{NTR}$ null mice exhibit anxiety-like behavior, and have difficulties coping with stress. Moreover, blockade of LTD by a LTD-specific peptide inhibitor also led to impaired stress-coping ability and decreased resiliency. It is possible that acute stress may enhance secretion of proBDNF, which through $p75^{NTR}$ facilitates LTD in the adult hippocampus. The proBDNF-$p75^{NTR}$-LTD may serve as a stress-coping pathway that aids the animal's recovery from stress reactions.

Regulation of intracellular trafficking of endogenous BDNF

Endogenous BDNF levels are extremely low, and specific antibodies are difficult to generate because of the high conservation among mammalian BDNFs. However, new tools, including a monoclonal antibody to the prodomain, and a gene-targeted knockin mouse expressing epitope-tagged *Bdnf* have facilitated quantitative detection of endogenous pro and mature BDNF (Yang et al., 2009). In addition these reagents permit analysis at the light and ultrastructural level of the intracellular localization of BDNF isoforms. Using these tools, proBDNF is expressed at significant levels at early postnatal stages (less than 4 weeks), whereas mature BDNF is the prominent isoform in the adult. In the juvenile brain, proBDNF immunoreactivity is diffusely observed in the developing hippocampus, in contrast to adults, where proBDNF immunoreactivity is largely restricted to the Mossy fibers of dentate granule neurons. In parallel, $p75^{NTR}$ immunoreactivity is most abundant in the juvenile hippocampus, and is markedly down regulated by 8 weeks. Collectively, these results suggest that the spatial and temporal expression of proBDNF and $p75^{NTR}$ are coordinated (Yang et al., 2009), with higher expression during the perinatal window when axonal outgrowth and synapse maturation is robust, and more localized expression during adolescence/adulthood.

These tools also permit localization of BDNF isoforms, across development, and following injury or stress paradigms, to distinct vesicular compartments in axons and dendrites. Several intracellular chaperone proteins, including carboxypeptidase E (Lou et al., 2005), which binds to the mature domain of BDNF, and sortilin, which binds to the prodomain of BDNF (Chen et al., 2005) have been identified, and are candidate proteins to potentially regulate intracellular localization of BDNF within neurons. The interactions of sortilin with proBDNF are important in dictating intracellular trafficking, as expression of dominant negative forms of sortilin lacking a cytoplasmic tail redirects BDNF from the regulated secretory pathway to the constitutive secretory pathway (Chen et al., 2005). This mouse model permits the dissection of the roles of sortilin and other BDNF-chaperone proteins in regulating intracellular trafficking of endogenous proBDNF/BDNF to axons or dendrites.

Summary

BDNF, the product of a single coding exon, elicits potent effects on neurons to regulate differentiation, synaptic plasticity, dendritic arborization, and furthermore modulates the establishment of neuronal circuits to regulate complex behaviors. Although models of BDNF haploinsufficiency have uncovered diverse cognitive and behavioral phenotypes, more recent studies, including those described above, underscore the multiple levels by which BDNF function can be manipulated across development, and in the setting of stress paradigms. The utilization of distinct promoter elements, the selective use of 3' UTRs to direct dendritic trafficking, the regulation of proBDNF to mature BDNF cleavage, and utilization of multiple intracellular chaperones to direct intracellular trafficking provide a diverse repertoire of regulatory mechanisms that permit central neurons to exquisitely and rapidly modulate BDNF expression, localization and synaptic delivery.

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