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Pro-Region of Neurotrophins: Role in Synaptic Modulation

Minireview

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Neurotrophins are synthesized first as precursors, followed by maturation through proteolytic removal of the "pro" region. Since pro- and mature neurotrophins elicit opposite functional effects by differential interactions with Trks and p75 receptors, extracellular cleavage represents a new way to control the synaptic functions of neurotrophins. A single nucleotide mutation in the pro-region appears to affect synaptic targeting and activity-dependent secretion of BDNF in hippocampal neurons. These results demonstrate new mechanisms by which neurotrophins regulate synaptic plasticity and memory function.

Neurotrophins, traditionally viewed as trophic proteins for neuronal survival and differentiation, have recently emerged as a new class of neuromodulators for synaptic transmission and plasticity, particularly in the central nervous system (CNS). These proteins are synthesized first as precursors of about 270 amino acids called pro-neurotrophins. The N-terminal fragment of ~120 amino acids, or the "pro-region," is then proteolytically cleaved in either trans-Golgi by furin or secretory granules by pro-protein convertases to form mature neurotrophins (Seidah et al., 1996). Neurotrophins are secreted constitutively in nonneuronal cells (Seidah et al., 1996), but secretion can be mediated by both constitutive and regulated secretion pathways in neurons and neuroendocrine cells (Mowla et al., 1999). Of particular interest is the fact that BDNF is secreted in an activity- and Ca^{2+}-dependent manner. The activity-dependent secretion of BDNF may be critically involved in controlling synaptic transmission and long-term synaptic plasticity and may represent an important mechanism underlying local and synapse-specific modulation by BDNF (Lu, 2003).

Until very recently, little attention has been paid to the pro-region of neurotrophins, which is thought to be removed intracellularly. Several recent papers have begun to shed light on the functional role of this region. The discovery that pro-neurotrophins are capable of interacting with p75 neurotrophin receptor (p75NR) with high affinity suggests that the pro-region may be critical for p75NR signaling (Lee et al., 2001). In another study, a single amino acid change in the pro-region of BDNF was found to affect synaptic targeting and activity-dependent secretion of the protein (Egan et al., 2003). Together, these new and unexpected results require a reevaluation of the mechanisms by which neurotrophins regulate synaptic transmission and plasticity.

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Pro-Region, Extracellular Cleavage, and Receptor Preferences

All neurotrophins are capable of binding to p75NR, and each also binds to a specific Trk receptor: NGF binds to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC. Binding of a neurotrophin to a specific Trk receptor activates its tyrosine kinase, leading to the activation of phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and phospholipase C-γ (PLC-γ) pathways. Interaction of neurotrophins with p75NR results in activation of very different signaling pathways such as increased NF-κB or c-*jun* N-terminal kinase activities. So far all the synaptic functions of neurotrophins have been attributed to the activation of Trk receptors but not p75NR. For example, regulation of neurotransmitter release at neuromuscular synapses by NT-3 requires TrkC and subsequent simultaneous activation of PI3K and PLC-γ/IP3 signaling pathways (Yang et al., 2001). In the hippocampus, inhibition of p75NR does not block BDNF regulation of presynaptic function or long-term potentiation (LTP) (Xu et al., 2000). Moreover, mutation of TrkB receptors at the PLC-γ docking site, but not the Shc site (required for activation of MAPK and PI3K), resulted in impairments in hippocampal LTP (Minichiello et al., 2002).

Recent work by Lee et al. has brought in an entirely new dimension to neurotrophin research and forced us to reconsider some well-established concepts and to reevaluate published data (Lee et al., 2001). There were three surprising findings. First, pro-neurotrophins may preferentially bind p75NR whereas mature neurotrophins may preferentially bind Trk receptors. Second, interaction of proNGF with p75NR induces apoptosis. This is in marked contrast to the interaction of mature neurotrophins with Trk receptors, which usually enhances cell survival. Third, proNGF and proBDNF can be cleaved in vitro by extracellular proteases, such as MMP7 and plasmin. Further, careful examination of previously published data indicates that pro-neurotrophins account for a significant proportion (40%–60%) of total neurotrophins secreted extracellularly, particularly in CNS neurons (Farhadi et al., 2000; Heymach et al., 1996; Mowla et al., 1999, 2001). While the endogenous extracellular proteases for pro-neurotrophins remain to be identified and the extent and role of extracellular cleavage are still being explored, it is clear that pro-neurotrophins are secreted extracellularly, where they bind to p75NR and produce biological functions distinct from those mediated by Trk receptors.

Given that pro- and mature neurotrophins interact with different receptors and have different biological functions, whether, where, and how pro-neurotrophins are cleaved may have profound implications in synaptic modulation. There are good reasons to believe that substantial amounts of pro-neurotrophins, particularly proBDNF, are secreted at synapses, either from the presynaptic terminals, from postsynaptic dendrites or spines, or from astrocytes ensheathing synaptic junctions. Compared with other neurotrophins, proBDNF is less efficiently processed by intracellular proteases (Mowla et al., 1999,

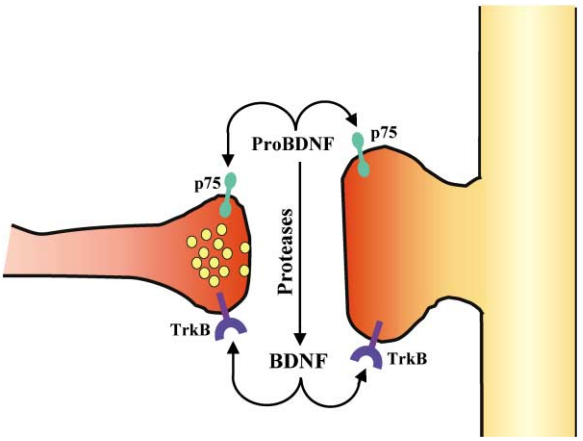

Figure 1. Extrasynaptic Cleavage of ProBDNF

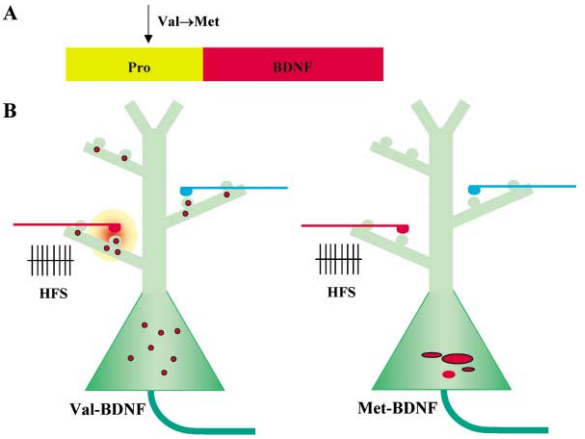

Figure 2. Cellular Phenotype of Val-Met Mutation in the Pro-Region of BDNF

2001). Schwann cells (and presumably astrocytes) secrete predominantly proNGF and proBDNF, suggesting that the intracellular cleavage system in glia is not very active (Mowla et al., 1999). In hippocampal neurons, BDNF mRNA is translocated to the dendrites, suggesting local synthesis of BDNF protein at synapses (Tongiorgi et al., 1997). The relatively primitive Golgi/secretory granule system in the dendrites may not be able to process proBDNF efficiently.

If pro-neurotrophins are secreted at synapses, they could regulate synaptic transmission and plasticity by several mechanisms. First, uncleaved pro-neurotrophins may act on p75NR on pre- or postsynaptic membranes and elicit effects distinct from those mediated by Trk receptors (Figure 1). While hundreds of papers have been published regarding the synaptic effects of neurotrophins, many have used quite high concentrations of neurotrophins that could have easily activated p75NR. Commercial recombinant neurotrophins have been shown to contain a substantial amount of pro-neurotrophins (Reinshagen et al., 2000). Thus, the effects observed in some of the studies may be attributed to the activation of p75NR, instead of Trks. This may explain why sometimes very different and even contradictory results have been obtained by different laboratories. Pro- and mature NGF has been shown to elicit opposite effects on cell survival (Lee et al., 2001). By analogy one might speculate that mature and pro-neurotrophins exert potentiating and depressing effects on synaptic transmission, respectively. Second, pro-neurotrophins are cleaved by proteases at synapses, converting inactive ligands (or a ligand for a different receptor) to active ligands for Trk receptors. This is particularly relevant at hippocampal synapses, where the BDNF-TrkB system is known to play a key role in regulating LTP. A good candidate protease is plasmin, which is expressed at synapses and can effectively cleave proBDNF at the appropriate site in vitro (Lee et al., 2001). The activity of plasmin is controlled by tissue plasminogen activator (t-PA), which has long been implicated in late-phase LTP and learning and memory (Baranes et al., 1998). Activity-dependent control of proteolytic cleavage may represent one way to ensure local and synapse-specific regulation by BDNF (Figure 1). Finally, the pro-regions themselves, or their proteolytic fragments, could serve as ligands for existing or new receptors. Such phenomena appear to be quite common for neuropeptides such as β-endorphin.

Pro-Region, Intracellular Sorting, Trafficking, and Activity-Dependent Secretion

Another function of the pro-region may be to control the intracellular processing, synaptic targeting, and/or secretion of neurotrophins. Some clues come from a recent study of a single nucleotide polymorphysm (SNP), which converts a valine (val) to methionine (met) at codon 66 in the 5′ pro-region of the human BDNF gene (Figure 2A; Egan et al., 2003). This SNP occurs in relatively high frequency and appears to be associated with Alzheimer's, Parkinson's, and bipolar disorders. Remarkably, human subjects with the BDNF met allele exhibit impaired hippocampal activity and memory function. The levels of n-acetyl aspartate (NAA), a putative in vivo measure of neuronal activity and synaptic abundance, were lower in the hippocampus of met allele subjects. Functional magnetic resonance imaging (fMRI) also revealed abnormal hippocampal activation during a memory task in these subjects. In a cohort of 641 subjects, those with the met/met genotype exhibited impaired episodic memory. At cellular levels, marked deficits were observed in the intracellular distribution, processing, and secretion of met-BDNF (Figure 2B). Rodent hippocampal neurons expressing val-BDNF-GFP (green fluorescence protein) exhibited a punctate distribution throughout cell bodies and dendrites, while those with met-BDNF-GFP formed large clusters in the perinucleus regions. While val-BDNF-GFP was frequently colocalized with the synapse markers, suggesting a targeting of BDNF-containing vesicles to the synapses, synaptic localization of met-BDNF-GFP was extremely rare. Further, regulated secretion of met-BDNF, as measured by neuronal depolarization with a brief exposure to high concentration of K^+, was markedly reduced as compared with that of val-BDNF. Interestingly, the val-met substitution did not affect constitutive secretion, which accounts for a small proportion of total secretion in hippocampal neurons.

While further experiments are required to establish

causal relationships, the combined in vivo and cell culture studies suggest that deficits in synaptic targeting and activity-dependent secretion of BDNF could lead to impairments in hippocampal activity and episodic memory in humans. The physiological implications of these findings are profound. Numerous studies in recent years have shown that BDNF regulates various synapses in a number of brain regions. It has been hypothesized that BDNF mediates various forms of activity-dependent synaptic plasticity, such as the formation of ocular dominance columns in the visual cortex and LTP in the hippocampus. How do diffusible factors such as BDNF achieve activity-dependent and synapse-specific modulation? Two requirements must be met. (1) BDNF must act locally and selectively to translate the effect of neuronal activity into structural and functional changes in specific synapses. At neuromuscular synapses in culture, BDNF-induced synaptic potentiation has been shown to be spatially restricted to micron range (Zhang and Poo, 2002). Local synthesis and/or secretion of BDNF are plausible mechanisms for the local and synapse-specific regulation. A prerequisite for such regulation is to target BDNF to synapses. (2) BDNF must act preferentially on active synapses without affecting the inactive ones nearby. One way to achieve this is to enhance the synaptic response to BDNF at an active synapse. High-frequency neuronal/synaptic activity has been shown to facilitate the insertion of BDNF receptor on the surface of hippocampal neurons (Du et al., 2000). Alternatively, BDNF may be secreted locally at the site of active synapses. BDNF is a sticky molecule, and its diffusion is further limited by the truncated TrkB molecules expressed on the dendritic surface of mature CNS neurons (Biffo et al., 1995). In cultured neurons, secretion of BDNF is dependent on stimulation frequency (Balkowiec and Katz, 2002). Assuming that activity-dependent secretion of BDNF is impaired in the hippocampus of human subjects with met allele, it is tempting to suggest that the activity-dependent, synaptic secretion of BDNF, rather than its overall concentration, is the key for synaptic plasticity.

A number of important questions remain. First, is the pro-region important for activity-dependent secretion of BDNF? The pro-regions of a number of neuropeptides have been shown to be critical in targeting peptides to regulated secretion pathways (Loh et al., 2002). For example, proopiomelanocortin (POMC) is secreted through the regulated pathway and is distributed in neuroendocrine cells as puncta in the neurites. Deletion of the N-terminal 25 amino acids in the pro-region results in constitutive secretion and diffuse staining in the perinuclear area. Similar results have been obtained for proenkephalin and chromogranin A. It has been hypothesized that the pro-region contains a "sorting signal" that interacts with carboxypeptidase E (CPE), a "sorting receptor" that directs polypeptides to the regulated pathway in neuroendocrine cells (Cool et al., 1997). Early studies indicated that the secretion of transfected NGF could be stimulated by cAMP analogs and deletion of various fragments in the pro-regions did not affect cAMP-induced secretion in PC12 cells (Heymach et al., 1996). In hippocampal neurons, however, NGF and NT-3 are diffusely distributed in peri-nuclear areas and are secreted via the constitutive pathway (Farhadi et al., 2000; Mowla et al., 1999). In contrast, BDNF is packaged in vesicles and secreted through the regulated pathway. When overexpressed, NT-3 and other neurotrophins have been shown to undergo regulated secretion, but these results were attributed to overexpression or mis-sorting. The fact that BDNF appears to be the only neurotrophin that is secreted through the regulated pathway (Farhadi et al., 2000; Mowla et al., 1999) suggests that the pro-region of BDNF may contain a unique structural element critical for its sorting to the regulated pathway. Sequence comparison reveals that a fragment containing val66 in the pro-region of BDNF is extremely well conserved among all species (from fish to human) but is very different from that of other neurotrophins including NT-4, which binds the same receptors as BDNF. When BDNF coding sequence was replaced by the NT-4 sequence using knockin techniques, the density of recycling-competent synapses as well as spontaneous transmission in cultured hippocampal neurons was greatly increased (Fan et al., 2000). Thus, the pro-region of NT-4 may target the protein to the constitutive pathway, leading to a global enhancement of all synapses. While the pro-region of BDNF may contain a unique structural determinant required for entry into the regulated pathway, it is unclear whether val66 is the critical residue for that domain. Curiously, the val-met mutation did not cause a compensatory increase in constitutive secretion, but results in an accumulation of met-BDNF aggregates in the peri-nuclear area (Egan et al., 2003). These results suggest that val66 is not involved in binary decision in sorting to constitutive or regulated pathway in the Golgi, but seems to be important for successful trafficking of BDNF from trans Golgi to secretory granules.

The second question remaining to be addressed relates to the molecular mechanisms underlying intracellular trafficking and synaptic targeting of BDNF-containing vesicles. The inability of met-BDNF-GFP to be transported to neuronal processes and localized to synapses suggests a possible role of the pro-region (Egan et al., 2003). The pro-region is critical for the correct folding of mature NGF, and presumably of mature BDNF as well. Correct folding of proteins in the Golgi is required for subsequent trafficking to the correct intracellular compartments. Whether the val-met substitution affects BDNF folding remains unknown. Nevertheless, the cellular phenotypes seen in met-BDNF-expressing neurons may be related: an unsuccessful trafficking of the protein within the regulated pathway may result in failure to target met-BDNF-containing vesicles to neuronal processes and synapses. Do BDNF-containing vesicles traffic to presynaptic terminals or postsynaptic dendrites? Biochemical studies have indicated that BDNF protein is enriched in a vesicular fraction in synaptosomes (Fawcett et al., 1997). Using innovative imaging techniques, Tsumoto and colleagues demonstrated an anterograde axonal trafficking of BDNF and its activity-dependent transfer from presynaptic to postsynaptic neurons (Kohara et al., 2001). Substantial evidence indicates that the majority of BDNF-containing vesicles are transported to postsynaptic dendrites and spines. The nature of these vesicles remains unclear. EM studies detected BDNF in large dense core vesicles (LDCV) in sensory neurons (Michael et al., 1997). In hippocampal

neurons, however, neither val- nor met-BDNF-GFP was colocalized with chromatogranin A, a marker for LDCV (Egan et al., 2003). It will be interesting to test whether BDNF is transported to pre- and postsynaptic sites through different types of secretory vesicles.

In summary, the pro-region of neurotrophins may play a critical role in their synaptic targeting and activity-dependent secretion at synapses. Once secreted, they may elicit entirely opposite effects on synapses depending on whether they are cleaved by extracellular proteases. These results not only force us to rethink and re-interpret some of the published results, but also open up an exciting new area of research on how neurotrophins control synaptic plasticity and cognitive functions in the CNS.

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