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Recent Advances in the Biology of BDNF And the Newly Identified Pro-Peptide

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ABSTRACT

Most growth factors are initially synthesized as precursors. To produce biologically active mature peptides, the pro-domain is cleaved by proteolytic processing. However, compared with mature bioactive growth factors, the biological roles of pro-domains are poorly understood. Recent new findings on brain-derived neurotrophic factor (BDNF), a growth factor in the brain that promotes neuronal survival, differentiation, and synaptic plasticity, have been reported. Interestingly, the pro-domain (pro-peptide) of BDNF is endogenously present and localized at presynaptic termini, where it surprisingly functions as a facilitator of long-term depression (LTD). Given that BDNF elicits synaptic transmission and long-term potentiation (LTP), BDNF and its pro-peptide might exert distinct roles in synaptic plasticity in the central nervous system (CNS). In addition to reports on the BDNF pro-peptide, we review recent literature on the role of BDNF in the peripheral nervous system (PNS), and in brain-body interactions following exercise. Together, these findings provide new insight into BDNF biology.

Neurotrophins and their biological actions

Neurotrophins (NTs), including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), exert distinct biological actions by binding to their cognate tyrosine kinase receptors (NGF to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC), as well as to the low-affinity neurotrophin receptor $p75^{NTR}$, which belongs to the tumor necrosis factor (TNF) family[1-3]. The discovery of NTs and their receptor components has provided new insight into the role of NTs in the formation of neuronal networks during nervous system development and synaptic plasticity, memory formation, learning, and memory in the adult brain[1,3-5]. Furthermore, specific neuronal populations require one or more NTs that act spatially and temporally[1,3,6]. For synaptic plasticity, NTs exert long- and short-term actions; long-term trophic actions depend on gene regulation, whereas short-term effects, including chemotrophic effects on developing neurons and synaptic events, are controlled by NT-mediated activation of intracellular effectors[5,7]. Since most of the existing literature focuses on the signaling mechanisms and transcriptional/translational control of NTs, we herein concentrate on post-translational aspects, in particular the effects of BDNF and its pro-domain (or pro-peptide) on synaptic plasticity in the brain.

Synthesis and Secretion of BDNF

Like other growth factors, BDNF is initially synthesized as a precursor composed of 270 amino acid residues. The pre-pro-protein includes a signal sequence, a pro-domain, and a mature domain. To

produce the mature form of BDNF, the N-terminal 120 amino acid pro-domain is cleaved by intracellular and/or extracellular proteases such as furin, pro-hormone convertase, and plasmin in the trans-Golgi and secretory granules, releasing BDNF[8]. The intracellular mechanisms of BDNF processing have been covered in several reports[9,10]. In neurons, the N-terminal pro-domain of precursor BDNF is cleaved in trans-Golgi and secretory granules, and BDNF is secreted through constitutive and/or neuronal activity-regulated secretion pathways in a neuronal activity- and $Ca2^{+}$-dependent manner (reviewed by Lessmann and Brigadski[8]). Thus, the activity-dependent secretion of BDNF is required for BDNF-dependent regulation of synaptic transmission and long-term synaptic plasticity [5]. The roles of BDNF on synapses are described below.

Modulation of Synaptic Function by BDNF

BDNF mRNAs are highly expressed in the brain, particularly in the hippocampus and cortex[11]. Expression of BDNF is controlled by neuronal activity and excitatory transmission[5]. Importantly, Dieni et al. (2012) demonstrated that BDNF is present in large, dense core vesicles within the excitatory presynaptic termini in the adult mouse hippocampus[12], implicating BDNF in synaptic transmission and plasticity, as subsequently proven by several groups. In cultured neurons, acute application of exogenous BDNF increased neuronal activity and synaptic transmission[13], and application of exogenous BDNF induced LTP in neonatal hippocampal slices[14]. Treatment with tropomyosin receptor kinase B (TrkB)-IgG, a fusion protein that scavenges endogenous BDNF, inhibited LTP in adult hippocampal slices[14], and recombinant BDNF rescued defective basal synaptic transmission and hippocampal LTP in BDNF knockout mice[15]. Furthermore, BDNF acts through TrkB, presynaptically but not postsynaptically, to modulate LTP[16]. Inhibition of $p75^{NTR}$ does not block BDNF regulation of presynaptic function and LTP in the hippocampus[16], and more importantly, mutation of TrkB receptor at the Phospholipase C (PLC)-γ docking site, but not at the SH2-containing collagen-related proteins (Shc) docking site, impaired hippocampal LTP[17], demonstrating the importance of TrkB-PLC-γ docking in BDNF-dependent LTP in the hippocampus.

The BDNF Pro-Domain (Pro-Peptide)

It is reported that BDNF controls numerous biological processes in the nervous system[1,3,5]. Thus, a thorough understanding of the role of the pro-domain could expand our appreciation of BDNF biology.

The BDNF Pro-Peptide is Localized in Presynaptic Dense Core Vesicles

A previous report showed that the BDNF pro-peptide/pro-domain acts as a molecular chaperone to assist the folding of BDNF[18]. We previously reported that a single-nucleotide polymorphism (SNP) that changes a valine to a methionine at codon 66 (Val66Met) in the pro-region of human BDNF affects memory function as well as the secretion of BDNF[19]. These results suggest that the BDNF pro-peptide may influence the biological actions of BDNF. To understand the functions of the BDNF pro-peptide, it is important to investigate and clarify the presence and localization of endogenous BDNF. Recently, Dieni et al. (2012) performed immunocytochemical and electron microscopy studies, and demonstrated that BDNF and its pro-peptide are both localized in dense core vesicles in excitatory presynaptic termini in the adult mouse hippocampus[12]. Notably, this report suggested an anterograde mode of action of BDNF in the central nervous system (CNS), distinct from the retrograde model derived from experiments with NGF in the peripheral nervous system (PNS). The authors further determined the amount of BDNF, precursor BDNF, and its pro-peptide, and showed that BDNF and its pro-peptide were approximately 10-fold more abundant than precursor BDNF in the adult mouse brain.

The BDNF Pro-Peptide is aA Facilitator of Hippocampal LTD

The BDNF pro-peptide, a portion of proBDNF, can enhance hippocampal LTD[6]. To induce LTD, low-frequency stimulation (LFS; 1 Hz, 900 pulses, 15 min) was applied to Schaffer collaterals of hippocampal slices prepared from 3- to 4-week-old mice, and field excitatory postsynaptic potential (fEPSP) slopes were recorded in the CA1 area. A 30 min treatment with the BDNF pro-peptide enhanced LTD without affecting basal transmission, and sub-nanomolar concentrations of the BDNF pro-peptide were sufficient for synapse facilitation. Importantly, application of the BDNF pro-peptide to *Bdnf*$^{-/-}$ hippocampal slices facilitated LTD, confirming that the BDNF pro-peptide acts independently of an interaction with endogenous BDNF. BDNF pro-peptide-dependent facilitation of hippocampal LTD requires activation of the $p75^{NTR}$ receptor and GluN2B/N-methyl-D-aspartate (NMDA) receptor subunit 2B-containing receptors. Moreover, the BDNF pro-peptide activates NMDA-induced α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor endocytosis, a critical mechanism for LTD expression. Together, these findings show that the BDNF pro-peptide stimulates synaptic plasticity, particularly LTD, and regulates a critical mechanism for promoting synaptic depression.

Interaction between BDNF and its Pro-Peptide Broadens the Intracellular Mechanisms and synaptic plasticity-related activity of BDNF

Comparison of the properties of BDNF and its pro-peptide indicated potential mechanistic and biological interactions. BDNF and its pro-peptide are basic and acidic, respectively,

with isoelectric points of 9.6 and 5.2[20], which suggests that they may bind in an electrostatic manner. We sought to investigate this putative interaction using surface plasmon resonance and biochemical methods[20]. Surprisingly, the BDNF pro-peptide binds to mature BDNF with high affinity, but not to other NTs. More interestingly, this interaction was enhanced at acidic pH compared with neutral pH. Thus, it is conceivable that stronger binding between BDNF and its pro-peptide may occur in acidic intracellular compartments such as trafficking vesicles, rather than in the extracellular space. To explore the physiological role of this interaction, we performed electrophysiological studies. Interestingly, when pre-incubated with BDNF, the BDNF pro-peptide completely attenuated the ability of BDNF to inhibit hippocampal LTD[6]. Thus, these results suggest that the BDNF pro-peptide, when co-released with BDNF, might modulate the availability of BDNF via a stable interaction in the extracellular space.

Novel insights: The Role of BDNF in the PNS and Brain-Body Integrity

Recently, numerous reports have revealed the roles and functions of BDNF in the PNS and the brain-body interface. BDNF expression is up-regulated in models of neuropathic pain, and BDNF enhances the ventral root potential induced by C-fiber stimulation, demonstrating the mechanical role of BDNF in the development of neuropathic pain[21]. However, injury to the sural nerve, which almost innervates skin, does not induce neuropathic pain. Recently, Zhou et al. (2010) indicated that sural nerve injury fails to produce neuropathic pain due to a limited amount of BDNF contained in the nerve[22]. Furthermore, in another recent study, Liu et al. (2017) demonstrated that TNF-alpha differentially regulates synaptic plasticity in the hippocampus and spinal cord via microglia-dependent mechanisms after peripheral nerve injury[23], suggesting a mechanical difference between chronic pain and memory deficit.

Exercise provides many beneficial effects to the human brain, including improving cognitive function, reducing the risk of Alzheimer's disease, and alleviating depression[24]. Recently, Ogborn et al. (2010) showed that 5 days of treadmill exercise elevated BDNF expression in the soleus more than in the medial gastrocnemius[25]. More recently, it was demonstrated that up-regulation of BDNF expression in the hippocampus after exercise is mediated by activation of the important metabolic mediator, peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1a (PGC-1a) and the previously identified muscle protein fibronectin type III domain containing 5 (FNDC5)[26], suggesting that the regulation of BDNF expression linking the brain and body after exercise is distinct from the neuronal activity-dependent up-regulation of BDNF expression that enhances synaptic function in the brain.

These findings provide new insight into the development of neuropathic pain and brain-body connections after exercise. Since both the BDNF pro-peptide and BDNF are derived from the same precursor, exactly how the BDNF pro-peptide contributes to the PNS and brain-body connections remains to be determined in future studies.

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