Impaired Secretion of Brain-Derived Neurotrophic Factor and Neuropsychiatric Diseases

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Abstract: Recent studies have elucidated mechanisms of brain-derived neurotrophic factor (BDNF) secretion, and impaired secretion of BDNF may be involved in the pathogenesis of several neuropsychiatric diseases. The huntingtin gene, for example, has been shown to regulate vesicular transport of BDNF, which may play a role in the neurodegeneration present in Huntington's disease. In animal studies, mice lacking calcium-dependent activator protein for secretion 2 (CADPS2), which is involved in the activity-dependent release of BDNF, showed several phenotypes including autistic behavior. A single nucleotide polymorphism that results in an amino-acid change (Val66Met) in the BDNF gene has been shown to cause a decline in the function of BDNF vesicular sorting and has been reported to be associated with behavioral and intermediate phenotypes (e.g., episodic memory) in humans. In this review, we introduce recent progress in the molecular mechanisms of BDNF secretion and discuss its possible role in the pathophysiology and treatment of neuropsychiatric diseases.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, has been implicated in a broad range of processes that are important for neuronal survival and synaptic plasticity in the central nervous system (CNS) [1-3]. Early in the 1950s, nerve growth factor (NGF) was discovered by Levi-Montalcini and Hamburger and Cohen [4,5] as a soluble factor that induced fiber outgrowth of chicken sympathetic neurons. Subsequently, Barde *et al*. [6] isolated BDNF, which was later found to be homologous to NGF [7], from pig brain as a neuronal survival factor. These discoveries motivated homology-based searches for additional family members of which there are currently a total of four in mammals – NGF, BDNF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). Additional members are conserved in fish – neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7) [8,9]. All neurotrophins are secreted from neuronal (partially glial) cells and bind to their receptors in an autocrine/paracrine manner. In the last two decades, a bulk of studies have suggested that neurotrophins, especially BDNF, are involved in the pathophysiology of neuropsychiatric diseases through their role in the regulation of synaptic efficacy (synaptic plasticity) and synaptogenesis in the CNS. In this review, we focus on recent findings of secretion mechanisms of BDNF and their relationship with neuropsychiatric diseases.

I. BIOLOGICAL FUNCTIONS OF BDNF

i. Survival and Synaptic Plasticity

Neurotrophins exert their biological effects through the binding of secreted homodimeric neurotrophins to two types of transmembrane receptor proteins: the tyrosine kinase Trk (tropomyosin-related kinase) receptors and the low affinity common neurotrophin receptor (p75NTR). Neurotrophins are expressed in a precursor form (pro-neurotrophins) and are proteolytically processed to a mature form. Mature neurotrophins preferentially bind to their specific Trk receptor: NGF to TrkA, BDNF and NT-4/5 to TrkB and NT-3 to TrkC. Pro-neurotrophins, however, bind to p75NTR with higher affinity than mature neurotrophins, although they have a lower affinity for Trk receptors [10]. Binding of neurotrophins to Trk receptors immediately generates receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain. Trk receptor phosphorylation activates intracellular signaling regulated by mitogen-activated protein kinase (MAPK), phosphoinositide-3 (PI3)-kinase/Akt and phospholipase C-γ (PLC-γ) pathways as well as several small G proteins, including Ras, Rap-1, and the CDC-42-Rac-Rho family [11-13]. These intracellular signaling cascades modulate expression of genes and are responsible for most of the long-term effects of neurotrophins related to neuronal growth, survival, and differentiation [14]. On the other hand, binding of pro-neurotrophins to p75NTR leads to antagonistic effects to Trk receptor signaling. Several of these p75NTR-dependent signaling are pro-apoptotic and can be suppressed by Trk receptor-initiated signaling [15,16]. The first evidence of a significant relationship between neurotrophins and synaptic plasticity was obtained by Lohof *et al.* [17]; exogenous BDNF and NT-3 increased synaptic efficacy at the *Xenopus* neuromuscular junction. Subsequently, these neurotrophins were shown to facilitate glutamatergic synaptic transmission, even in the hippocampus of the mammalian CNS [18-20]. There is now substantial evidence implicating BDNF in activity-dependent long-term synaptic plasticity [21,22]. The neurotrophin-binding Trk receptor activates many kinds of signaling pathways that promote neuronal survival and synaptic efficiency, although it is still unclear how the complex signaling pathways are

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systematically integrated to generate many biological functions.

ii. Activity-Dependent Expression of Neurotrophins

Among neurotrophins, BDNF shows the most ubiquitous expression in the developing and adult mammalian brain. BDNF expression levels are increased dramatically during the first few weeks of postnatal development. Expression of neurotrophins in neurons is linked to neuronal activity. BDNF and NGF mRNA levels are rapidly increased by seizure activity in the hippocampus and the cerebral cortex [23-25]. In contrast, blockade of visual input causes rapid down-regulation of BDNF mRNA in the rat visual cortex of dark-reared animals [26]. A similar phenomenon has been found in cultured neurons. The introduction of glutamate with high concentration potassium-induced depolarization increase levels of BDNF and NGF mRNA, while blockade of neuronal activity with γ-aminobutyric acid (GABA) decreases such levels [27,28].

iii. Processes of BDNF Secretion

BDNF is synthesized as a 32 kDa precursor protein (proBDNF) and proteolytically cleaved to generate the mature BDNF (13 kDa). The synthesis of the pro-BDNF occurs at the rough endoplasmic reticulum (ER). Following this, pro-BDNF is transported to the Golgi apparatus and concentrated in membrane stacks of the *trans*-Golgi network (TGN). Finally, BDNF-containing vesicles bud off the TGN to eventually transport to the releasing sites. Recent studies clarified some of the details of BDNF vesicular sorting. Specifically, the pro-region of BDNF has been implicated as a regulator of BDNF sorting to secretory vesicles [29]. Moreover, fusing the pro-region of BDNF to NT-4, which is rarely sorted into secretory vesicles, allowed NT-4 to sort more efficiently into specific vesicles [30]. These data support the importance of the BDNF pro-region as a potential target to help guide secretory granules. Furthermore, binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is also important for sorting into secretory vesicles of the regulated pathway [31]. Sortilin, a trans-membrane protein, has also been implicated in the sorting of BDNF to secretory glanules. Sortilin is expressed in secretory granules and interacts specifically with the pro-region of BDNF. Interestingly, the truncated form of sortilin results in missorting of BDNF to the secretory vesicles [32].

It is still controversial as to where and how pro-neurotrophins are processed into mature neurotrophins in the CNS. Originally, it had been thought that pro-neurotrophins are prototypically cleaved by furin and pro-protein convertases (PCs) in the TGN or in secretory granules before secretion [33]. However, recent studies have indicated that a considerable amount of BDNF is secreted in the pro-form from neurons. Released pro-BDNF is subsequently processed to mature BDNF extracellularly by proteases such as plasmin or matrix metalloproteinases [34,35]. More recently, however, it was shown that pro-BDNF is rapidly converted into mature BDNF intracellularly and almost all BDNF was secreted as the mature form from hippocampal neurons [36].

iv. Constitutive and Regulated Secretion

Secretion of neurotrophins is classified into "constitutive" and "regulated" pathways, depending on whether the secretion occurs spontaneously or in response to neuronal activity, respectively. In hippocampal neurons, BDNF appears to be sorted primarily into the regulated pathway [37-39]. In the regulated pathway, BDNF-containing vesicles are transported into either presynaptic axon terminals or post-synaptic dendrites along microtubules for activity-dependent secretion [40-43]. Recently, Lessmann and colleagues conducted an elegant study that provided the long-awaited understanding of BDNF secretion. The activity-dependent postsynaptic secretion of neurotrophins critically depends on Ca^{2+} influx *via* ionotropic glutamate receptors or voltage-gated Ca^{2+} channels, Ca^{2+} release from internal stores, activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), and intact protein kinase A (PKA) signaling. Trk signaling and activation of Na^{+} channels, on the other hand, are not required for BDNF secretion [44-46]. Furthermore, recent reports suggest that the Golgi apparatus exists in dendrites as well as the cell soma, and have gone so far as to identify a local BDNF secretory pathway in neuronal dendrites [47,48]. Future works may reveal more details concerning the secretory systems of neurotrophins at the subcellular level and that may be more complex and dynamic than we can presently imagine.

II. IMPAIRED SECRETION OF BDNF AND NEUROPSYCHIATRIC DISEASES

i. Huntington's Disease

Huntington's disease (HD) is a fatal, dominantly inherited, neurodegenerative disease that usually presents during midlife. It is characterized by relatively selective degeneration of striatal neurons which lead to psychiatric, cognitive and motor dysfunction. Polyglutamine expansion (polyQ) in the protein huntingtin (htt) is thought to be the principal mechanism for the neuronal toxicity in HD. Recently, evidence has indicated the possible link between HD and BDNF. Wild type htt plays a role as a transcription factor and facilitates expression of BDNF [49]. Furthermore, htt has been implicated in BDNF-containing vesicle transport. Mutant (PolyQ)-htt perturbs post-Golgi trafficking of BDNF in the regulated secretory pathway, though it does not influence the constitutive pathway [50,51]. Conversely, the exogenously transfected BDNF gene generated increased BDNF levels and TrkB signaling in the striatum, which resulted in improved symptoms in HD model mice [52]. These findings suggest that mutation of htt reduces levels of BDNF in the striatum by inhibiting gene expression and perturbing anterograde transport of BDNF-containing vesicles from cortex to striatum. Therefore, the development of therapies focused on the reduction of BDNF release should be important for future studies.

ii. Rett Syndrome

Rett syndrome (RTT) is an X-linked disorder characterized by arrested neurological development and subsequent cognitive decline. Methylation of DNA in vertebrates occurs preferentially on cytosine residues of dinucleotides in which the cytosine is followed by a guanine residue (CpGs). Meth-

ylated CpGs bind a variety of proteins. One of these proteins, methyl-CpG binding protein 2 (MeCP2), has been implicated in the long-term silencing of gene expression. Inactivating mutations in MeCP2 is caused in the majority of cases with Rett syndrome. Chen *et al*. showed that MeCP2 selectively binds to the BDNF promoter III and represses expression of BDNF [53]. Membrane depolarization triggers the calcium-dependent phosphorylation and release of MeCP2 from the BDNF promoter III, thereby facilitating transcription [54]. A conditional BDNF transgene increased BDNF expression in the MeCP2 mutant brain, which resulted in rescue of locomotor defects, recovery of electrophysiological deficits, and extension of lifespan in MeCP2 mutant animals [55]. Although MeCP2 null mice exhibited a slightly decreased content of BDNF in some brain areas, mutant neurons demonstrated equivalent secretion levels of BDNF compared to wild-type in response to high-frequency electrical stimulation [56]. Furthermore, BDNF expression in MeCP2 null neurons was significantly improved by chronic ampakine treatment, which was administered to facilitate AMPA receptor activation [57]. These results suggest that the expression of BDNF is still plastic in the MeCP2 null condition and manipulating the BDNF level or the BDNF signaling pathways may provide therapeutic opportunities for RTT patients.

iii. Autism

Autism is a severe neurodevelopmental disorder with a childhood onset, characterized by profound disturbances in socialization, language skills, communicative, and behavioral functions. BDNF is expressed abnormally in individuals with autism and, as a result, may be involved in the pathogenesis of autism [58,59]. Elevated levels of BDNF and NT4/5 measured by archived neonatal blood samples of autistic patients were reported [60]. Elevation of BDNF was also reported in a study of 18 Japanese children with autism compared with controls [61]. These findings suggest that excess BDNF during childhood may be involved in the neurobiological abnormalities observed in autism. The specific molecular mechanisms involving BDNF and autism remain unknown, though one report suggests that genetic changes in autistic individuals may account for altered neurotrophin levels [62]. Ca^{2+}-dependent activator protein for secretion 2 (CAPS2/CADPS2) is a secretory granule-associated protein that is abundant at the parallel fiber terminals of granule cells in the mouse cerebellum and is involved in the release of BDNF and NT-3. The human CAPS2/CADPS2 gene is located on chromosome 7q31.32 within a critical autism susceptibility locus 1 (AUTS1). CAPS2 knock-out mice demonstrate autistic-like behavioral phenotypes and deficient release of BDNF and NT-3. Moreover, phosphorylation of Trk receptors is decreased in the cerebellum, which may play a role in the pronounced impairment of cerebellar development and function, including neuronal survival, differentiation and migration of postmitotic granule cells, that these mice exhibit [63]. Although there have been few reports suggesting the relation between autism and BDNF secretion, further investigation may result in novel insights.

iv. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures. Various studies have shown that BDNF increases neuronal excitability and is up-regulated in areas implicated in epileptogenesis. Seizure activity increases expression of BDNF mRNA and protein, and recent studies have shown that interfering with BDNF signal transduction inhibits the development of the epileptic state *in vivo* [64]. Half of all drug-resistant individuals experience seizure control with dietary manipulation, such as isocaloric substitution of carbohydrates with fats and protein referred to as the 'ketogenic diet'. Daley *et al*. reported that an inhibitor of glycolysis is shown to have antiepileptic effects in the rat kindling model, which may be related to NADH-dependent regulation of BDNF expression [65]. This result may explain how the 'ketogenic diet' treatment works. Although it is unclear whether the up-regulation of BDNF is the cause or the consequence of epilepsy, the reduction of BDNF expression or BDNF signaling can be a useful tool for the treatment of epilepsy.

v. Psychiatric Disorders

Mood and anxiety disorders are the most common psychiatric diseases. BDNF has been implicated in these disorders, because decreased levels of BDNF in the hippocampus are correlated with stress-induced depressive behaviors [66]. Other studies also showed decreased plasma levels of BDNF in patients with major depression [67]. Many classes of antidepressants, including selective serotonin reuptake inhibitors, significantly increase BDNF mRNA expression in the hippocampus and prefrontal cortex [68,69]. The time course of such increase is consistent with the slow onset of therapeutic effects of antidepressants. More recently, striking evidence for the involvement of TrkB-dependent neurogenesis in the antidepressant effect has been reported. Mice lacking TrkB in the hippocampal neuron progenitor cells had impaired neurogenesis and proliferation induced by antidepressant treatment. These mice also demonstrated increased anxiety-like behavior and decreased sensitivity to antidepressants [70,71]. Taken together, BDNF may play a key role in the brains of recovering patients during antidepressant treatment [72,73].

Many reports have isolated the possible association between BDNF levels and schizophrenia in several brain regions [74]. However, results from these studies are contradictory in that some demonstrate decreased BDNF levels in the postmortem brain or serum, while others report that the BDNF level in patients was not significantly different from that in normal controls [75]. Moreover, samples used in each experiment differ in age, species (rodents, primates, human) and regions (i.e., hippocampus, frontal cortex, CSF and blood) [76]. Although there have been many studies examining the possible role of BDNF in schizophrenia, integrated knowledge concerning this has not been produced. Despite this, the neurobiological vulnerability paradigm remains an attractive concept, supporting that increased susceptibility may be a consequence of reduced expression of BDNF (neurotrophins) at a certain point of life [77].

In the region encoding BDNF's pro-region, a SNP was identified at amino acid 66 (Val66Met). Egan and colleagues reported that the met allele was associated with decreased episodic memory and abnormal hippocampal activation as assayed with fMRI in human subjects [29]. Furthermore, neurons transfected with met-BDNF-GFP showed lower

depolarization-induced secretion, while constitutive secretion was unchanged. Met-BDNF-GFP failed to localize to secretory granules or synapses [29]. Following this, a number of association studies of this polymorphism with psychiatric disorders have been done. Unexpectedly, the Met66 allele, which reduces BDNF release, has been suggested to be protective against developing bipolar disorder [78], although this association was not confirmed by large-scale studies [79,80]. The Met66 allele has also been implicated in other disorders like anorexia [81]. Future research is required to assess how the Val66Met is associated with particular psychiatric disorders.

CONCLUSIONS

The biological mechanisms of neurotrophins are critically important for neuronal functions that affect brain functions and behavior. Growing evidence has implicated BDNF in the pathophysiology of many neuropsychiatric diseases. Genetic variations leading to deranged expression or secretion due to altered transcription, vesicular sorting, vesicular trafficking and secretion of BDNF seem to play an important role in several neuropsychiatric diseases and related behavioral phenotypes. In order to develop treatment strategies for these diseases through targeting neurotrophins and their receptors, however, clarification of more detailed mechanisms is needed. Studies that reveal not only an increase/decrease in expression of neurotrophins, but also accurate spatiotemporal secretion profiles of neurotrophins are necessary.

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