



PROBDNF BIOLOGY AND EMERGING ROLES IN THE CNS: THE UNEXPECTED JOURNEY OF PRONEUROTROPHINS

Margarita Arango-Lievano, Agustin Anastasia, Freddy Jeanneteau

► To cite this version:

Margarita Arango-Lievano, Agustin Anastasia, Freddy Jeanneteau. PROBDNF BIOLOGY AND EMERGING ROLES IN THE CNS: THE UNEXPECTED JOURNEY OF PRONEUROTROPHINS. Brain-Derived Neurotrophic Factor (BDNF): Therapeutic Approaches, Role in Neuronal Development and Effects on Cognitive Health, 2015, 978-1-63483-761-3. hal-02368091

HAL Id: hal-02368091

<https://hal.science/hal-02368091>

Submitted on 18 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

PROBDNF BIOLOGY AND EMERGING ROLES IN THE CNS: THE UNEXPECTED JOURNEY OF PRONEUROTROPHINS

***Margarita Arango-Lievano^{1,*}, Agustin Anastasia²
and Freddy Jeanneteau¹***

¹Dep. Physiology, Institut de génomique fonctionnelle; Inserm U1191,
CNRS UMR5203- Montpellier, France

²Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC-CONICET-
UNC - Córdoba, Argentina.

ABSTRACT

Neurotrophins are synthesized as precursors (proneurotrophins) with a N-terminal prodomain and a C-terminal mature domain. Classically, neurotrophins must be cleaved off their prodomains to be operational. That is to promote neuronal and non-neuronal cell survival, growth and differentiation via rapid signaling routes and long lasting changes in gene transcription. This view came with the assumption that the proforms of all neurotrophins are inactive. But since the early 2000s, several laboratories driven by the group of Dr. Barbara L. Hempstead *revealed* that the proforms are signaling molecules as well. Proneurotrophins can be secreted and bind specifically and with high affinity to the p75 neurotrophin receptor (p75^{NTR}) as receptor. Recent studies on Brain Derived Neurotrophic Factor (BDNF) demonstrated that proBDNF effects via p75^{NTR} opposes the functions of mature BDNF through its cognate tropomyosin related kinase B (TrkB) receptor. These antagonistic effects include synaptic plasticity (depression versus potentiation), dendritic spine maintenance (elimination versus growth) and cell fate (death versus survival). In this chapter we will review proBDNF intracellular and extracellular processing, transport, signaling mechanisms, as well as its biological function in health and disease. We will focus on the effect of a common human polymorphism in the BDNF gene inducing a Val66Met substitution that causes structural remodeling of the prodomain promoting a gain of function of this peptide independently of mature BDNF. Given their features, neurotrophins have been implicated in numerous

* Corresponding author: margarita.arango@igf.cnrs.fr.

neurodegenerative diseases and psychiatric disorders in which the Met66 allele human carriers are more vulnerable than the Val66 carriers.

INTRODUCTION

Long considered to be only necessary for the biosynthetic folding of mature BDNF, the prodomain has emerged as a unique signaling molecule functionally distinguishable from its mature counterpart. Henceforth, it is considered that three functional ligands can be derived from the BDNF gene: proBDNF which signals through a complex of p75^{NTR} and sortilin or SorCS2 (sortilin-related VPS10 domain containing receptor 2), the Met66 prodomain which also activates a p75^{NTR}/SorCS2 receptor complex, and the mature BDNF which signals via TrkB receptors to elicit independent cellular responses. Here, we first review BDNF processing from the proform to mature BDNF and prodomain, with special focus on the role the latter. In a second part, we organize recent evidence demonstrating the signaling properties of proBDNF focusing on secretion, specific binding properties to its receptors and its biological effect on the central nervous system. Finally, we gather the recent evidence suggesting that the prodomain itself is an independent signaling molecule.

1. FROM ONE GENE TO THREE LIGANDS: THE PROCESSING OF PROBDNF

1.1. Synthesis and Sorting

Like all neurotrophins, BDNF is synthesized as a large precursor called proBDNF. This precursor is approximately a 32kDa peptide that is glycosylated in the N-terminal prodomain region [1, 2]. Following synthesis in the endoplasmic reticulum (ER), proBDNF is directed to the secretory pathway. ProBDNF can be cleaved to prodomain and mature BDNF in the Golgi apparatus or in secretion vesicles but, during early development, substantial amounts of proBDNF can escape processing. Contrary to the neurotrophins NGF (nerve growth factor), NT3 (neurotrophin 3) and NT4 (neurotrophin 4) that are secreted in a constitutive manner, BDNF can be secreted via the constitutive or the activity-dependent secretory pathways [2]. Sorting likely occurs in the trans Golgi network (TGN) where proBDNF interact with the type 1 receptor sortilin [3]. Sortilin is a member of a group of receptors containing a vacuolar sorting protein 10 (VPS10) domain that act like chaperones to target their cargo protein to different cellular compartments [4]. By interacting with the prodomain, sortilin acts as a chaperone for intracellular trafficking and directs proBDNF into dense-core vesicles destined to the activity dependent secretory pathway [2, 5]. Yet, interaction with sortilin is also necessary to engage BDNF toward the lysosomal pathway [6]. Two mechanistic frameworks explain how proBDNF is directed toward secretory or degradation routes. First, targeting of sortilin and its proBDNF cargo to the lysosomal pathway may rely on the cleavage of sortilin by ADAM 10 [6]. Second, targeting of sortilin and its proBDNF cargo to the secretory pathway may require physical interaction with the huntingtin interacting protein 1 (HAP1) [7]. A common human single nucleotide polymorphism (SNP) on the prodomain of BDNF is

associated with memory impairment and increased incidence of neuropsychiatric disorders [8, 9]. This SNP leads to a nucleotide change from a guanine to an adenine at position 196 (G196A) that results in a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met). The SNP was reported to alter the transport of BDNF mRNA transcripts to dendrites for local synthesis of BDNF [10]. The Val66Met substitution also disrupts the interaction between the proBDNF and sortilin, resulting in a reduced activity dependent secretion of BDNF [3, 8].

1.2. Cleavage of ProBDNF into Mature BDNF

Mature BDNF is a 14KDa peptide with well-characterized trophic and plasticity abilities that have been extensively reviewed elsewhere [11, 12]. The prodomain is a 15.5kDa protein recently found to be very abundant in the hippocampus [5, 13, 14]. Mature BDNF and the prodomain result from proteolytic cleavage of proBDNF. Several proteases have been shown to cleave proBDNF both in the intracellular and in the extracellular milieu. In some culture conditions, the latter seems to be less prominent than the intracellular cleavage. Indeed, inhibition of the main extracellular proteolytic enzymes did not affect the levels of the secreted prodomain, suggesting that most of the processing occurred intracellularly [13]. On the other hand, it has been shown that neuronal activity controls the ratio of extracellular proBDNF/mature BDNF by regulating the secretion of extracellular proteases. All these intra- and extracellular enzymes cleave proBDNF at distinct but interdependent sites, generating biochemically undistinguishable mature BDNF and prodomain species. No distinct biological functions of the mature BDNF cleaved by one or the other proteases have ever been reported to our knowledge. The following are the enzymes which have been described to process proBDNF.

1.2.1. *Furin and other proconvertases*

Following synthesis in the ER, proBDNF can undergo processing directly in the Golgi apparatus by furin or within secretory vesicles of the regulated pathway by other proconvertases [2]. The cleavage of proneurotrophins by furin occurs at the consensus sequence R-X-K/R-R, (RVRR in proBDNF) to produce mature neurotrophins and prodomains. Furin cleaves proBDNF at Arg 128 of the consensus site. The mutation of this consensus site from RVRR to RVAA[15] or MVLRL[16] has been instrumental to produce a cleavage resistant proBDNF insensitive to furin and plasmin, and has been exploited to produce recombinant uncleavable proBDNF to demonstrate its biological activity in vitro and in vivo [15-18]. Other proconvertases, such as PACE4, PC5, and PC7, have been shown to process proBDNF in mature BDNF and prodomain utilizing the same RVRR site [19-21].

1.2.2. *Plasmin*

When proBDNF escape intracellular cleavage by furin or other proconvertases, it can be found in the dense-core vesicles destined to the secretory pathway. The existence of secreted proBDNF was the subject of an intense debate that we will discuss later in this chapter, but if secreted, proBDNF can be converted into mature BDNF and prodomain by extracellular proteases. The most prominent of these proteases is plasmin, which is the product of the

activation of the inactive plasminogen by tissue-type plasminogen activator (tPA). The specific plasmin cleavage site of proBDNF was identified within the consensus furin-cleavage sequence of BDNF, but occurring after Arg¹²⁵ rather than Arg¹²⁸ of the RVRR sequence [22]. Interestingly, a SNP has been identified at position 125, which induces an arginine substitution for a methionine that would potentially prevent plasmin processing of proBDNF [16]. Several lines of regulation are implicated in this reaction as plasminogen to plasmin conversion is antagonized by Plasminogen activator inhibitor (PAI1), and PAI1 depends on vitronectin to remain active [23]. Furthermore tPA is an extracellular serine protease which secretion is stimulated by neuronal activity [24]. The fact that both proBDNF/BDNF/prodomain containing vesicles and tPA are secreted in an activity dependent fashion raises questions about regulation of bio-available tPA. For instance, a reduction of tPA secretion may be enough to favor proBDNF signaling and vice and versa.

1.2.3. Matrix metalloproteinases

ProBDNF can be processed by selected matrix metalloproteinases (MMPs) such as MMP-3 and MMP-7 [25]. Moreover, it has been shown that MMP-9 converts proBDNF into mature BDNF after kindled seizures in the hippocampus [26].

2. PROBDNF SIGNALING

In order to function as signaling molecule like most neuropeptides, proBDNF must (i) be secreted, (ii) bind to a specific receptor, and (iii) trigger biological functions. Below we will discuss the evidence of how proBDNF fulfill these conditions.

2.1. Secretion of ProBDNF

Whether proBDNF can be secreted as a signaling molecule has been the subject of a very intense debate. Observing proBDNF directly in the extracellular milieu of mice is difficult due to the nanomolar concentrations of BDNF and the lack of antibodies that are specific and sensitive enough to identify proBDNF in such quantities. The first evidence of proBDNF secretion was obtained from a pituitary derived cell line (ATt20) infected with a rabies virus expressing recombinant human BDNF. In these conditions proBDNF as well as mature BDNF were found on the culture media of cells [2]. These conditions were useful to demonstrate that it is possible for cells to secrete proBDNF, but were very far from physiological as they rely on overexpression, and did not answer whether endogenous proBDNF could be secreted by neurons. Other cell lines including HEK293 and endothelial cell lines infected with adenovirus encoding for BDNF were used to demonstrate that proBDNF is released in the culture media and readily cleavable extracellularly when exposed to recombinant plasmin. On the contrary, the quantities of extracellular proBDNF increased after treatment of cells with the protease inhibitor aprotinin [25]. Interestingly, high frequency neuronal activity was capable of inducing the secretion of proBDNF alongside with the mature BDNF on hippocampal neurons transduced with a lentivirus for the overexpression of BDNF [27]. Despite the demonstration of activity-dependent secretion, the yield of extracellular proBDNF remained low compared to the mature BDNF. The results suggested

that (i) extracellular proBDNF is unstable readily cleavable by activity-dependent extracellular proteases [27] and that (ii) extracellular proBDNF could have escaped intracellular cleavage due to the overexpression experimental approach. This controversy was supported by evidence arguing against the neuronal secretion of endogenous proBDNF [28]. To avoid overexpression, this study used neuronal cultures from a knock-in mouse featuring a Myc tag fused to BDNF (N terminal region) allowing the expression of the BDNF gene under the endogenous promoters and detection of proBDNF and BDNF by very sensitive reagents against the Myc tag. They found that although endogenous levels of mature BDNF and proBDNF are detectable in neuronal cell lysates, only the mature BDNF was observed in the extracellular space after neuronal activation via NMDA receptors [28]. Notably, this study did not supplement the conditioned media with inhibitors against plasmin or matrix metalloproteinases to preserve secreted proBDNF in culture media. Moreover, they did not utilize mitotic inhibitors to avoid glial cell growth, which are a source of a variety of extracellular proteases. A subsequent study addressed this issue combining a different knock-in mouse to label mature BDNF and proBDNF with a C-terminal HA tag and an antibody raised specifically against proBDNF. Endogenous levels of proBDNF were detected in the conditioned media of hippocampal neuronal cultures in the absence of glial cells, and treated with a plasmin inhibitor [29]. In cultures, at least part of proBDNF secretion was activity dependent [29]. Whether the secretion of proBDNF has a functional relevance is addressed in the next sections

2.2. Receptors for ProBDNF

The affinities of proneurotrophins and mature neurotrophins for different receptors were first explored for proNGF and NGF by Lee et al. [25]. The authors showed that proNGF and mature NGF can bind to P75 neurotrophin receptor ($p75^{NTR}$) and tropomyosin receptor kinase A (TrkA), respectively. ProNGF is a high affinity ligand for $P75^{NTR}$ (Kd of 1nM) and mature NGF has a high affinity for both TrkA (Kd 1nM) and $p75^{NTR}$ (Kd 2nM). On the other hand, proNGF has no affinity for TrkA but binds to $p75^{NTR}$ (Kd of 0.2 nM) hinting that proNGF could have an independent signaling mechanisms through $p75^{NTR}$ [25]. Sortilin was found to be essential for proNGF/ $p75^{NTR}$ activation making this receptor the first co-receptor to be identified for proneurotrophins [30]. Sortilin binds specifically to the prodomain region of proNGF with high affinity (Kd= 5nM). When sortilin and $p75^{NTR}$ are co-expressed they exhibit a synergetic effect on proNGF internalization rather than a simple additive effect [30]. Sortilin, $p75^{NTR}$ and proNGF co-immunoprecipitate after crosslinking suggesting the possibility that proNGF could bind to sortilin and $p75^{NTR}$ at the same time [30]. Such a dual receptor system was later replicated for proBDNF which can bind to $p75^{NTR}$ utilizing its mature moiety and to sortilin by its prodomain region [31]. The affinities of proBDNF for its receptors were determined using purified recombinant proBDNF and immobilized sortilin. Sortilin binds with high affinity (Kd of 0.4 nM) to the prodomain region of proBDNF whereas $p75^{NTR}$ affinity for proBDNF showed a Kd of 20 nM. Interestingly, ProBDNF does not bind to the TrkB receptor [17]. The sortilin- $p75^{NTR}$ co-receptor system appears to be functionally relevant as binding of proBDNF to sortilin was necessary for $p75^{NTR}$ mediated cell death induced by this ligand. Abundant in the nervous system [32], sortilin is predominantly present on intracellular membranes [33], which limits its capacity to transduce

proBDNF signal with p75^{NTR}. Therefore, it is important to understand how sortilin localization to the plasma membrane is regulated. The mammalian homologue of p75^{NTR}, NRH2 (PLAIDD or NRADD) interacts with sortilin. This interaction reduces its lysosomal degradation, thus favoring the proportion of surface to intracellular sortilin and its association with p75^{NTR} and proBDNF [34]. It is important to note that while the first co-receptor to be identified for proneurotrophin signaling through p75^{NTR} was sortilin, other members of the VSP10 family such as SorCS2 can also act as co-receptors [35]. An independent report confirmed this findings demonstrating that SorCS2 binds to the prodomain region of NGF, BDNF and NT3, as well as to p75^{NTR} [36].

2.3. Biological Roles of ProBDNF

2.3.1. Cell death and survival

While the pro-survival effect of BDNF on central and peripheral neurons is well documented, the role of proBDNF on neuronal survival is cell-type specific. Initial experiments in cultured superior cervical ganglion (SCG) neurons [17], where p75^{NTR} expression is substantial, revealed that application of recombinant uncleavable proBDNF triggers neuronal cell death [17]. Additionally it has been reported that application of cleavage resistant proBDNF elicits cell death only on granular neurons from the cerebellum but not in hippocampal pyramidal neurons or cholinergic neurons from the basal forebrain that also express high levels of p75^{NTR} [16, 37]. In the cell types where proBDNF promotes cell death, p75^{NTR} is necessary for this function, [17, 37]. In SCG neurons, proBDNF binding to sortilin is also implicated in the apoptotic mechanism [17]. ProBDNF, through its interaction with p75^{NTR} and sortilin, has also been shown to promote caspase-dependent death of motor neurons in culture [38]. On cerebellar granular neurons c-Jun N-terminal kinases (JNK), caspase 3 and Rac-GTPases activation seem to be implicated in the apoptotic mechanism [37]. These examples illustrate that while p75^{NTR} expression is necessary for proBDNF induced cell death it is not sufficient to determine the cell response to the propeptide.

2.3.2. Synaptic potentiation and depression

Mature BDNF is necessary for the establishment of the late phase of long-term potentiation (L-LTP). This has been demonstrated in hippocampal neurons of BDNF hemizygous mice after high frequency stimulation (HFS) establishing that the deficit on the L-LTP can be rescued by the application of recombinant mature BDNF [18]. This effect mimicked that of the tPA/plasminogen complex previously identified as an activity-dependent protease that converts proBDNF into mature BDNF. Other than BDNF, the NMDA receptor is a prime target of tPA/Plasminogen important for synaptic plasticity [39, 40]. Interestingly, the enhancement of NMDA signaling by tPA may be responsible for the increase of extracellular mature BDNF levels resulting from neuronal activity [41]. Unequivocally, both plasminogen deficient mice and tPA knockout presented L-LTP deficits that are rescued by bath application of mature BDNF [18]. In light of these arguments, it is particularly interesting to note the opposing effect of proBDNF on synaptic plasticity. Application of cleavage resistant proBDNF to hippocampal slices enhanced NMDA receptor dependent long-term depression (LTD) following low frequency stimulation (LFS) [18]. As expected,

this effect of proBDNF is mediated by $p75^{\text{NTR}}$, which is expressed on presynaptic terminals, postsynaptic spines and dendritic shafts of the CA1 hippocampal region, consistent with its role on proBDNF-mediated LTD [42]. Hippocampal slices from $p75^{\text{NTR}}$ depleted mice failed to develop LTD after LFS despite normal basal synaptic transmission and neurotransmitter release. Likewise, hippocampal slices from wild type mice that were pretreated with $p75^{\text{NTR}}$ blocking antibodies failed to develop LTD after LFS. These results are consistent with the developmental expression of LTD and $p75^{\text{NTR}}$, higher in early life (3 weeks) when induced-LTD is stronger, than in mature animals (8weeks).

2.3.3. Neuronal differentiation

Neurite retraction and reduction on dendritic spine density in response to proBDNF application occur in DRG [43] and SCG [17] neurons as well as in hippocampal [13, 16], cortical [43], and cholinergic neurons [16]. Interestingly, proBDNF can induce neurite retraction most likely through a $p75^{\text{NTR}}$ dependent mechanism, as proBDNF has no effect on neuronal cultures from $p75^{\text{NTR}}$ deficient mice [43]. The cellular mechanism implicated in proneurotrophins-induced neurite remodeling was first described for proNGF in cultured hippocampal neurons [35]. ProNGF application induces a retraction of the growth cone of a subset of hippocampal neurons expressing $p75^{\text{NTR}}$ within minutes of treatment. The effect is mediated by a complex of $p75^{\text{NTR}}$ and its co-receptor SorCS2 through the RhoA/Rac GTPase system that impinged on the polymerization of the actin cytoskeleton [35]. A similar signaling mechanism converging on small GTPases activation and actin depolymerization was described for growth cone retraction of DRG neurons upon proBDNF incubation. However, the co-receptor for $p75^{\text{NTR}}$ was not studied in this investigation [43].

2.3.4. In vivo evidence for a biological role of proBDNF

Most studies investigating the possible biological role of proBDNF have been executed in vitro using relatively high concentrations of the recombinant uncleavable protein. Despite demonstrating a consistent effect of the proform on reducing dendritic arborization and enhancing LTD in hippocampal neurons as well as enhancing apoptosis in DRG neurons, the actual relevance of these findings in vivo was still unclear. To answer this question the laboratory lead by Dr. Barbara L. Hempstead came up with an elegant genetic animal model in which, the BDNF gene is replaced by a cleavage resistant mutant (RVRR to RVAA mutation on the cleavage site). The heterozygous version of this knock-in mice (proBDNF/+) expresses one copy of the cleavage-resistant proBDNF and one copy of the wild type BDNF, resulting in a haplo-insufficiency of mature BDNF and an overexpression of proBDNF. To discriminate between the effects of mature BDNF haplo-insufficiency and those triggered by the increased levels of proBDNF, all experiments were conducted comparing the proBDNF/+ heterozygote mice with BDNF +/- mice and wild type controls [15]. Using this model, they confirm hippocampal atrophy on proBDNF/+ mice that is significantly stronger than in BDNF +/- mice. By Scholl analysis, they confirmed a reduction in dendritic arborization of granule neurons in the hippocampus that was present in juvenile animals (P30) and became more noticeable in older animals (P105). The effect of BDNF haploinsufficiency was significantly lower than that of proBDNF overexpression supporting an in vivo role of this proneurotrophin. ProBDNF has no effect on granular neuron arbor complexity in $p75^{\text{NTR}}$ deficient mice, confirming the requirement of this receptor for proBDNF actions in vivo. Furthermore, proBDNF induces a significant decrease on dendritic spines density on

hippocampal neurons suggesting a role of this ligand on spine elimination or in reducing spinogenesis. Consistent with a loss of dendritic spines, proBDNF/+ mice exhibits impairments of basal synaptic transmission at the Shaffer collateral synapses. ProBDNF/+ mice presents a deficit on LTP following tetanic burst stimulation (TBS) but have normal HFS-induced LTP, which demonstrate a proBDNF specific effect on LTP that was not previously identified with recombinant proBDNF approaches. Finally, confirming previous reports proBDNF/+ mice have enhanced LFS LTD [15]. The study carried out by the Hempstead group represents a crucial advance in our understanding of proBDNF roles in the structural and physiological properties of the hippocampus.

The use of proBDNF knock-in mice genetic tool will be instrumental to shed light on how proBDNF can affect major functional aspects of the brain such as memory acquisition and retention, and susceptibility to neuropsychiatric diseases such as depression. Indeed several studies reviewed below suggest that proBDNF signaling could be implicated in disease states.

2.3.5. Is proBDNF implicated in disease states?

BDNF is a neurotrophin, which deregulation has been implicated in several neuropsychiatric and neurodegenerative disorders, ranging from depression, cognitive impairment, Alzheimer's, Parkinson's and Huntington's disease among others. The emergence of proBDNF as an independent signaling molecule bears the question of whether the regulation of proBDNF processing and its cognate receptor complex expression could also mediate some aspects of the nervous system pathology. To this regard, proBDNF signaling has been associated with seizures-induced cell death in mice. Several studies have reported an increased expression of proneurotrophins and p75^{NTR} following drug-induced status epilepticus (SE) in rodents [44, 45]. SE induction provokes neuronal cell death in the hippocampus through a p75^{NTR}-mediated mechanism [45, 46]. SorCS2 and both proNGF and proBDNF are upregulated in the hippocampus of rats following SE [45] while in mice, proNGF is not [44], suggesting that these proneurotrophins could mediate p75^{NTR} dependent cell death. While proneurotrophins appear to be important in the early phase of these models of SE, mature BDNF signaling through TrkB is also implicated as inactivation of this receptor's activity following SE is sufficient to inhibit development of chronic spontaneous seizures [47]. Moreover, SE induces a TrkB to p75^{NTR} receptor switch that contributes to the seizure-induced cell death; however, these studies propose that mature BDNF, but not proBDNF, are responsible for p75^{NTR} activation and neurodegeneration in this pathological condition [48, 49].

So far, only one study has explored the use of proBDNF as a potential biomarker for disease with negative results. Yoshida et al. explored whether mature BDNF or proBDNF could be used as biomarkers for major depressive disorder (MDD). Using specific antibodies for proBDNF and mature BDNF, they tested peripheral blood of MDD patients and age and gender matched controls, and found a significant decrease on mature BDNF but no changes on proBDNF [50]. The neuronal upregulation of p75^{NTR} is a feature of several other pathological conditions including ischemia [51] and Alzheimer disease [52] but whether proBDNF is the ligand on those conditions is still to be determined.

3. PRODOMAIN SIGNALING

The prodomain of BDNF itself is detectable *in vivo* and has been recently found to be a secreted independent ligand [5, 13, 14] from mature BDNF and proBDNF. Below we describe the main findings of this newly described active peptide.

3.1. Secretion of the BDNF Prodomain

According to the sorting and cleavage system of BDNF described before, the prodomain of BDNF is foreseen to be released from neurons. A careful electron microscopy study of the adult hippocampus revealed that the prodomain is indeed present in presynaptic secretory dense-core vesicles along with the mature form of BDNF [5]. This finding suggests that the prodomain is likely to be released in the synaptic cleft, co-secreted with mature BDNF and unprocessed proBDNF. A recent report confirmed that the prodomain in isolation is detected extracellularly [13]. The experimental approach of this report utilized cultured hippocampal neurons in conditions to reduce glia contamination, collected the conditioned media, and detected the endogenous prodomain secreted utilizing specific monoclonal antibodies. In this study, the prodomain was secreted in an activity-dependent manner after depolarization of the cultured neurons. Incubation of the hippocampal neuron cultures with a plasmin inhibitor and/or MMP inhibitor II (which inhibits MMP1, 3, 7 and 9) to prevent extracellular cleavage of secreted proBDNF did not alter significantly the levels of the secreted prodomain in the media, in basal conditions or after depolarization [13]. Interestingly, both the Val66 and Met66 prodomains can be secreted after depolarization with potassium chloride; however, the levels of secreted Met66 prodomain are significantly lower as compared with the Val66 prodomain. This finding is in agreement with previous studies which showed that the Val66Met polymorphism leads to a decrease in the trafficking of BDNF to secretory vesicles and the subsequent impairment of activity-dependent release of mature BDNF [3, 8, 53].

3.2. Biological Roles of the Prodomain

As the BDNF prodomain has been very recently identified as an independent ligand, its effects on neuronal survival have not yet been reported. Next, we will review the reported biological functions of the prodomain.

3.2.1. Neuronal morphology

In a recent study, Anastasia et al. explored whether the prodomain elicit an independent biological function [13]. To do so, they explored the structure and properties of the most frequent prodomain (with a valine in position 66 in 75% of the global population) and the prodomain carrying Val66Met substitution (present in 25% of the population). This substitution within the prodomain sequence is the result of a common human polymorphism associated with memory impairment and associated risk of depression and anxiety [8, 9, 53, 54]. As described above, the proposed mechanism of action for this substitution in neuropsychiatric disorders susceptibility is a loss of function of mature BDNF, which is the

consequence of a decrease activity dependent secretion [8]. Anastasia et al. proposes an additional mechanism to explain the polymorphism impact on human behavior, in which the Val66Met substitution alters the conformation of the prodomain and confers a gain of function to the Met66 prodomain affecting neuronal morphology. Specifically, they found that the Met66 prodomain (but not the Val66) is capable of interacting with SorCS2 to induce growth cone retraction in hippocampal neurons. Interestingly this new function of the prodomain appears to be dependent on p75^{NTR} expression as the Met66 prodomain is inert on p75^{NTR} deficient cells [13]. But how p75^{NTR} signaling is required for the Met66 prodomain is still unclear as neither the Val66 nor the Met66 prodomains interact with this receptor [13].

3.2.2. *LTD and LTP*

In light of the facilitation of LTD induced by proBDNF, Mizui et al. explored the implication of the isolated prodomain in hippocampal neuronal plasticity, more precisely on LFS-induced LTD. They demonstrate that the BDNF but not the NGF prodomain facilitates LFS-induced LTD, an effect that also depends on p75^{NTR} [14]. Interestingly, the Met66 prodomain shows the opposite effect and inhibits LFS-induced LTD, but the implication of p75^{NTR} on the Met66 prodomain effect was not explored. These results are consistent with a previous study describing a deficit on LFS-induced LTD in hippocampal slices of BDNF^{met/met} mice [55], which suggest that the prodomain could be responsible for this effect. The LFS protocol elicits NMDA dependent LTD that relies, in part, on trafficking of AMPA receptor subunits. Mizui et al. demonstrate that the Val66 prodomain facilitates LTD by promoting the surface expression of GluN2B NMDA receptor subunit and the endocytosis of AMPA receptors, while the Met66 prodomain blocks it. These results imply a general effect of the Met66 prodomain on NMDA signaling [14]. The deficient NMDA-dependent LTP of the BDNF^{met/met} [55] mice supports also this hypothesis. Unfortunately the effect on the Met66 prodomain in hippocampal LTP has not yet been reported.

Until now, the effect of the Val66Met polymorphism was mainly explained by a loss of function of mature BDNF consequence of a decrease on activity dependent BDNF secretion [53]. These recent publications exploring the biological function of the BDNF prodomain independently of mature BDNF and proBDNF, describe gains of functions for the Val66Met polymorphism, raising the question of which BDNF ligands are contributing more to the phenotypic characteristics of the Met66 human carriers.

CONCLUSION

Substantial evidence supports the idea that proBDNF function goes beyond being a synthesis precursor for mature BDNF. Therefore, factors such as synthesis, sorting, release and processing of proneurotrophins, as well as their cognate receptors availability, could be potent modulators of homeostasis in the nervous system. The concomitant expression of proBDNF and p75^{NTR} in the hippocampus during early post-natal periods and its subsequent decrease in adulthood is a good example of developmental shift between functions of the proBDNF, mature BDNF and its prodomain. The implication of proBDNF in pathological states remains to be demonstrated.

REFERENCES

- [1] Mowla, SJ; et al. Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J Biol Chem*, 2001, 276(16), 12660-6.
- [2] Mowla, SJ; et al. Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *J Neurosci*, 1999, 19(6), 2069-80.
- [3] Chen, ZY; et al. Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *J Neurosci*, 2005, 25(26), 6156-66.
- [4] Nykjaer, A; Willnow, TE. Sortilin: a receptor to regulate neuronal viability and function. *Trends Neurosci*, 2012, 35(4), 261-70.
- [5] Dieni, S; et al. BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *J Cell Biol*, 2012, 196(6), 775-88.
- [6] Evans, SF; et al. Neuronal brain-derived neurotrophic factor is synthesized in excess, with levels regulated by sortilin-mediated trafficking and lysosomal degradation. *J Biol Chem*, 2011, 286(34), 29556-67.
- [7] Yang, M; et al. Precursor of brain-derived neurotrophic factor (proBDNF) forms a complex with Huntingtin-associated protein-1 (HAP1) and sortilin that modulates proBDNF trafficking, degradation, and processing. *J Biol Chem*, 2011, 286(18), 16272-84.
- [8] Egan, MF; et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 2003, 112(2), 257-69.
- [9] Dincheva, I; Glatt, CE; Lee, FS. Impact of the BDNF Val66Met polymorphism on cognition: implications for behavioral genetics. *Neuroscientist*, 2012, 18(5), 439-51.
- [10] Chiaruttini, C; et al. Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. *Proc Natl Acad Sci U S A*, 2009, 106(38), 16481-6.
- [11] McAllister, AK; Katz, LC; Lo, DC. Neurotrophins and synaptic plasticity. *Annual review of neuroscience*, 1999, 22, 295-318.
- [12] Binder, DK; Scharfman, HE. Brain-derived neurotrophic factor. *Growth factors*, 2004, 22(3), 123-31.
- [13] Anastasia, A; et al. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun*, 2013, 4, 2490.
- [14] Mizui, T; et al. BDNF pro-peptide actions facilitate hippocampal LTD and are altered by the common BDNF polymorphism Val66Met. *Proc Natl Acad Sci U S A*, 2015, 112(23), E3067-74.
- [15] Yang, J; et al. proBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. *Cell Rep*, 2014, 7(3), 796-806.
- [16] Koshimizu, H; et al. Multiple functions of precursor BDNF to CNS neurons: negative regulation of neurite growth, spine formation and cell survival. *Mol Brain*, 2009, 2, 27.
- [17] Teng, HK; et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci*, 2005, 25(22), 5455-63.
- [18] Pang, PT; et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science*, 2004, 306(5695), 487-91.

- [19] Wetsel, WC; et al. Disruption of the expression of the proprotein convertase PC7 reduces BDNF production and affects learning and memory in mice. *Proc Natl Acad Sci U S A*, 2013, 110(43), 17362-7.
- [20] Marcinkiewicz, M; Savaria, D; Marcinkiewicz, J. The pro-protein convertase PC1 is induced in the transected sciatic nerve and is present in cultured Schwann cells: comparison with PC5, furin and PC7, implication in pro-BDNF processing. *Brain Res Mol Brain Res*, 1998, 59(2), 229-46.
- [21] Seidah, NG; et al. Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Lett*, 1996, 379(3), 247-50.
- [22] Gray, K; Ellis, V. Activation of pro-BDNF by the pericellular serine protease plasmin. *FEBS letters*, 2008, 582(6), 907-10.
- [23] Mou, X; Peterson, CB; Prosser, RA. Tissue-type plasminogen activator-plasmin-BDNF modulate glutamate-induced phase-shifts of the mouse suprachiasmatic circadian clock in vitro. *The European journal of neuroscience*, 2009, 30(8), 1451-60.
- [24] Shin, CY; Kundel, M; Wells, DG. Rapid, activity-induced increase in tissue plasminogen activator is mediated by metabotropic glutamate receptor-dependent mRNA translation. *J Neurosci*, 2004, 24(42), 9425-33.
- [25] Lee, R; et al. Regulation of cell survival by secreted proneurotrophins. *Science*, 2001, 294(5548), 1945-8.
- [26] Mizoguchi, H; et al. Matrix metalloproteinase-9 contributes to kindled seizure development in pentylenetetrazole-treated mice by converting pro-BDNF to mature BDNF in the hippocampus. *J Neurosci*, 2011, 31(36), 12963-71.
- [27] Nagappan, G; et al. Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proc Natl Acad Sci U S A*, 2009, 106(4), 1267-72.
- [28] Matsumoto, T; et al. Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nat Neurosci*, 2008, 11(2), 131-3.
- [29] Yang, J; et al. Neuronal release of proBDNF. *Nat Neurosci*, 2009, 12(2), 113-5.
- [30] Nykjaer, A; et al. Sortilin is essential for proNGF-induced neuronal cell death. *Nature*, 2004, 427(6977), 843-8.
- [31] Teng, KK; et al. Understanding proneurotrophin actions: Recent advances and challenges. *Dev Neurobiol*, 2010, 70(5), 350-9.
- [32] Petersen, CM; et al. Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. *J Biol Chem*, 1997, 272(6), 3599-605.
- [33] Nielsen, MS; et al. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO J*, 2001, 20(9), 2180-90.
- [34] Kim, T; Hempstead, BL. NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. *EMBO J*, 2009, 28(11), 1612-23.
- [35] Deinhardt, K; et al. Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Sci Signal*, 2011, 4(202), ra82.
- [36] Glerup, S; et al. SorCS2 regulates dopaminergic wiring and is processed into an apoptotic two-chain receptor in peripheral glia. *Neuron*, 2014, 82(5), 1074-87.
- [37] Koshimizu, H; et al. Distinct signaling pathways of precursor BDNF and mature BDNF in cultured cerebellar granule neurons. *Neuroscience letters*, 2010, 473(3), 229-32.
- [38] Taylor, AR; et al. Motoneuron programmed cell death in response to proBDNF. *Dev Neurobiol*, 2012, 72(5), 699-712.

- [39] Samson, AL; Medcalf, RL. Tissue-type plasminogen activator: a multifaceted modulator of neurotransmission and synaptic plasticity. *Neuron*, 2006, 50(5), 673-8.
- [40] Nicole, O; et al. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. *Nat Med*, 2001, 7(1), 59-64.
- [41] Rodier, M; et al. Exogenous t-PA administration increases hippocampal mature BDNF levels. plasmin- or NMDA-dependent mechanism? *PLoS One*, 2014, 9(3), e92416.
- [42] Woo, NH; et al. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat Neurosci*, 2005, 8(8), 1069-77.
- [43] Sun, Y; et al. ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. *PLoS One*, 2012, 7(4), e35883.
- [44] VonDrán, MW; et al. p75NTR, but not proNGF, is upregulated following status epilepticus in mice. *ASN Neuro*, 2014, 6(5).
- [45] Volosin, M; et al. Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *J Neurosci*, 2008, 28(39), 9870-9.
- [46] Troy, CM; Friedman, JE; Friedman, WJ. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J Biol Chem*, 2002, 277(37), 34295-302.
- [47] Liu, G; et al. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. *Neuron*, 2013, 79(1), 31-8.
- [48] Unsain, N; Montroull, LE; Masco, DH. Brain-derived neurotrophic factor facilitates TrkB down-regulation and neuronal injury after status epilepticus in the rat hippocampus. *J Neurochem*, 2009, 111(2), 428-40.
- [49] Unsain, N; et al. Status epilepticus induces a TrkB to p75 neurotrophin receptor switch and increases brain-derived neurotrophic factor interaction with p75 neurotrophin receptor: an initial event in neuronal injury induction. *Neuroscience*, 2008, 154(3), 978-93.
- [50] Yoshida, T; et al. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *PLoS One*, 2012, 7(8), e42676.
- [51] Kokaia, Z; et al. Focal cerebral ischemia in rats induces expression of P75 neurotrophin receptor in resistant striatal cholinergic neurons. *Neuroscience*, 1998, 84(4), 1113-25.
- [52] Mufson, EJ; Kordower, JH. Cortical neurons express nerve growth factor receptors in advanced age and Alzheimer disease. *Proc Natl Acad Sci U S A*, 1992, 89(2), 569-73.
- [53] Chen, ZY; et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, 2006, 314(5796), 140-3.
- [54] Hajek, T; Kopecek, M; Hoschl, C. Reduced hippocampal volumes in healthy carriers of brain-derived neurotrophic factor Val66Met polymorphism: meta-analysis. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, 2012, 13(3), 178-87.
- [55] Ninan, I; et al. The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *J Neurosci*, 2010, 30(26), 8866-70.